(A) BODIPY as a versatile fluorophore
(B) Synthesis of PNAs via Staudinger reaction

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Submitted to the Department of Chemistry

in partial fulfillment of the requirements for the degree of

Master of Science

Brock University

St. Catharines, Ontario

Abstract

(A) In recent years, 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (BODIPY) fluorophores have attracted considerable interest due to their unique photochemical properties. However detailed studies on the stability of BODIPY and analogues under acidic and basic conditions have been lacking. Thus the stability of a series of BODIPY analogues in acidic (di- and trichloroacetic acid) and basic (aqueous ammonium hydroxide) conditions was investigated using ^{11}B NMR spectroscopy. Among the analogues tested, 4,4-diphenyl BODIPY was the most stable under the conditions used in the experiments. It was found that reaction of 4,4-dimethoxy BODIPY with dichloroacetic acid gave mixed anhydride 4,4-bis(dichloroacetoxy) BODIPY in good yields. Treatment of the latter mixed anhydride with alcohols such as methanol and ethanol in the presence of a base afforded corresponding borate esters, whereas treatment with 1,2-diols such as ethylene glycol and catechol in the presence of a base gave corresponding cyclic borate esters. Furthermore treatment of 4,4-difluoro-8-methyl-BODIPY with secondary amines in dihalomethane resulted in carbon–carbon bond formation at the *meso*-methyl position of BODIPY via Mannich-type reactions. The resulting modified BODIPY fluorophores possess high fluorescent quantum yields. Five BODIPY analogues bearing potential ion-binding moieties were synthesized via this Mannich-type reaction. Among these, the BODIPY bearing an aza-18-crown-5 tether was found to be selective towards copper (II) ion, resulting in a large blue shift in absorption and sharp fluorescent quenching, whereas aza-15-crown-4 analogue was selected towards fluoride ion, leading to effective florescent quenching and blue shift.

(B) Peptide nucleic acids (PNA), as mimics of natural nucleic acids, have been widely applied in molecular biology and biotechnology. Currently, the preparation of PNA oligomers is commonly achieved by a coupling reaction between carboxyl and amino groups in the presence of an activator. In this thesis attempts were made towards the synthesis of PNA through the Staudinger ligation reactions between *C*-terminal diphenylphosphinomethanethiol thioesters and *N*-terminal α-azido PNA building blocks.

Acknowledgement

I would like to express my deepest appreciation to the people who provide help, support and guidance through my graduate study at Brock University. First and foremost, I would like to thank my supervisor and mentor Professor Tony Yan for his guidance and supervision to make the thesis possible. He treated me with great patience so that I have learned a lot in the lab throughout the years. I could not have imagined having a better advisor and mentor for my graduate study.

I would like to thank the supervisory committee members, Professors Atkinson and Metallinos for their valuable advice and insightful comments throughout the years.

My sincere thanks also go to my fellow labmates: Ningzhang Zhou, Qiang Wang, Dhruval Joshi, Jia Li, Ravi Yalagala and Nazanin Heidari, for the stimulating discussions and all the fun we have had. Also I thank Tim Jones and Razvan Simionescu for their assistance in collecting data for mass spectrometry and NMR spectroscopy and Professor Antai Wu for help in screening BODIPY compounds.

Lastly, I would like to thank the Natural Sciences and Engineering Research Council of Canada for funding this work.

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Ac	acetyl
AcOH	acetic acid
BODIPY	borondipyrromethene difluoride
$CDCl_3$	deuterated chloroform
d	doublet
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCA	dichloroacetic acid
DCC	1,3-dicyclohexyl carbodiimide
DMAP	4-dimethylaminopyridine
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
EI	electron impact
ESI	electrospray ionization
EtOAc	ethyl acetate
FAB	fast atom bombardment
h	hour
m	multiplet
M	molar concentration
MeOH	methanol

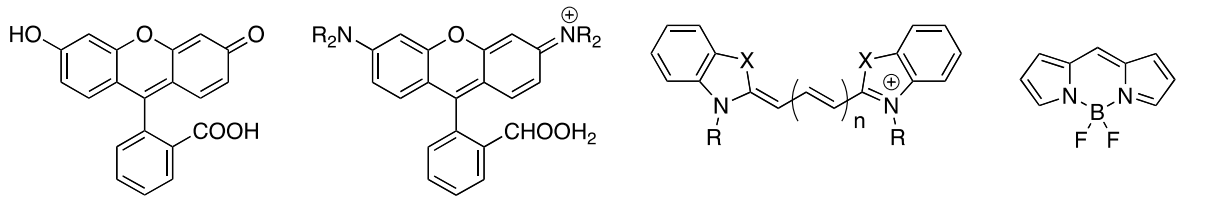
min	minute
NMR	nuclear magnetic resonance
Ph	phenyl
$^{i}Pr_2EtN$	*N,N*-diisopropylethylamine
RNA	ribonucleic acid
t	triplet
TCA	trichloroacetic acid
TFA	trichloroacetic acid
THF	tetrahydrofuran
U	uridine

Chapter 1 BODIPY as a versatile fluorophore

1.1 Introduction to fluorophores

Fluorescence is a property of a substance that allows it to emit light upon exposure to at a higher frequency light. Since Sir John Frederick William Herschel reported the first observation of fluorescence from a quinine solution in sunlight in 1845,[1] fluorescence has been widely applied in diverse practical usages, such as chemical sensors, mineralogy, fluorescent labeling, dyes, and biological detectors. Fluorescence spectroscopy, fluorescence imaging and fluorescence indicators are indispensable tools in various fields of modern science and medicine, including clinical diagnostics, molecular biology, biochemistry, materials science, and environmental chemistry.[2] In biological assays and medical diagnosis in particular, fluorescence methods have attracted increasing interests over the past few years.[3] Specific fluorescent reporters have been designed to combine with recognition sites, which enable researchers to detect components of complex biomolecular assemblies with high sensitivity.[4] Thus, fluorescence methods have become alternatives to radioactive labeling, especially for sensing and visualization of analytes inside living cells.[5]

Among the numerous fluorophores reported to date, the principal classes are based on fluorescein, rhodamine, cyanine and difluoro-bora-indacene family (4,4-difluoro-4-bora-3a-azonia-4a-aza-*s*-indacene, abbreviated hereafter as F-BODIPY) (Figure 1-1).



Fluorescein skeleton **Rhodamine skeleton** **Cyanine skeleton** **BODIPY skeleton**

Figure 1-1. Organic fluorescent dyes structures

1.2 Introduction to borondipyrromethene difluoride (BODIPY)

Among various types of highly fluorescent dyes, the BODIPY-class fluorophore has earned increasing interest as a versatile heterocyclic skeleton fluorophore. The first members of BODIPY derivatives were reported by Alfred Treibs and Franz-Heinrich Kruezer at 1968.[6] However, more attention was given to the BODIPY based-dyes and their potential applications in biological labeling in the late 1980s.[7, 8] Biological labeling with BODIPY was not implemented until 1989 when BODIPY labeled ligands were first synthesized as highly selective D1 and D2 dopaminergic probes.[9] In the past 2-3 decades, due to their unique photochemical and photophysical properties, there have been enormous research interests on the BODIPY fluorophores, which is reflected by the number of patents and journal articles on the syntheses and applications of BODIPY-based dyes. Over 700 patents and 1000 journal articles had been published by 2006.[10]

The IUPAC name of BODIPY is 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene by analogy to the all-carbon tricyclic *s*-indacene ring system (Figure 1-2). Hence, rules for numbering any substituents on the BODIPY core structure are the same as the carbon polycycle. On the other hand, BODIPY can also be regarded as an analogue of 'dipyrrin core', and thus, the terms α-, β-, and *meso*-position are applicable to both BODIPY and dipyrrin systems (Figure 1-2).[11]

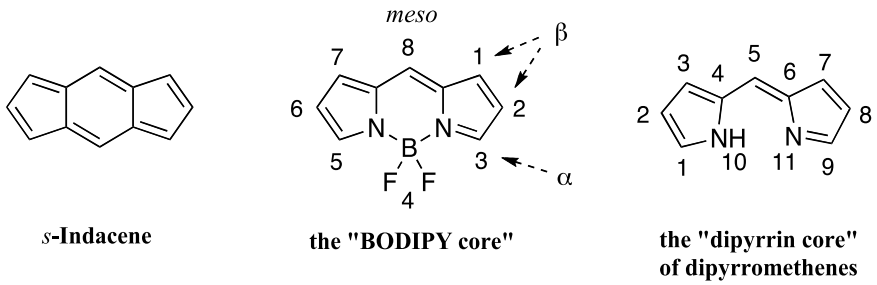


Figure 1-2. Numbering schemes used for the dipyrromethenes and BODIPY framework derived from indacene.

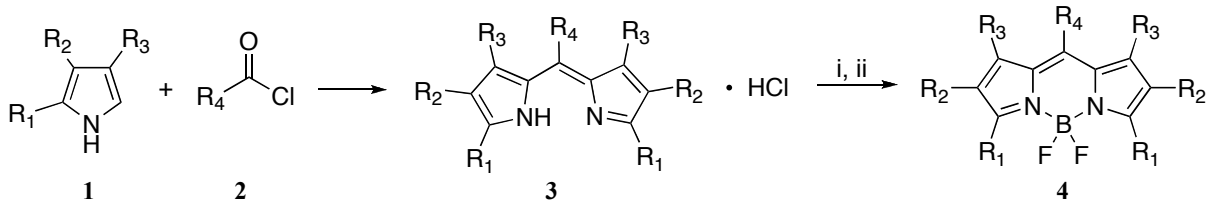
BODIPY-based fluorophores exhibit extraordinary optical properties, such as high molar extinction coefficients, high fluorescent quantum yields, sharp excitation and emission peaks, and small Stock's shifts.[12] Moreover, they are relatively stable thermally, chemically and photochemically, while they are fairly insensitive to solvent polarity and pH.[12] Therefore, BODIPY-based fluorophores are superior to other fluorophores in some applications,[12, 13] and have shown dramatic growth in popularity.

In general, the majority of BODIPY-based dyes are substituted. Fully unsubstituted BODIPY core was not prepared until 2009 by three independent groups.[14] The main obstacle in the synthesis of this fully unsubstituted BODIPY core structure relates to the instability of the fully unsubstituted dipyrromethene precursor.[14]

BODIPY derivatives are also associated with certain drawbacks that limit their applications. Among these, narrow Stoke's shift, poor water solubility and lack of functional groups for conjugation are commonly cited limitations.[15] Through the introduction of various substituents on the 2-, 3-, 5-, 6-, and *meso*-positions, properties of BODIPY can be modulated to suit various applications.

1.3 General synthesis of BODIPY

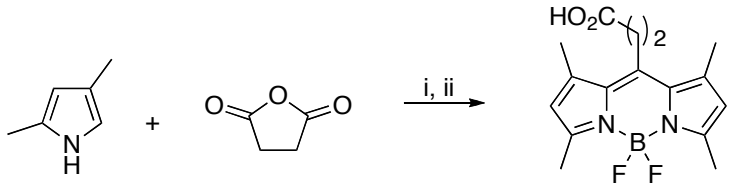
A number of approaches have been reported for the synthesis and modification of the BODIPY fluorophore. The most well-known route for the preparation of the BODIPY fluorophore begins with condensation of acyl chlorides **2** with pyrroles **1** to yield the unstable dipyrromethene hydrochloride salt intermediates **3**, which subsequently undergoes complexation with borontrifluoride in the presence of a base such as triethylamine and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (Scheme 1-1).[16] This approach is particularly suited for the synthesis of symmetric BODIPY dyes **4**. Isolation of the unstable intermediate dipyrromethene hydrochlorides **3** is quite difficult, however, the intermediates **3** become easier to handle with the increasing number of the C-substituents. Nevertheless, these intermediates **3** are rarely isolated during the synthesis of BODIPY.



1 **2** **3** **4**

Scheme 1-1: *Reagents and conditions*: i) NEt_3, $^{i}Pr_2EtN$ or DBU; (ii) $BF_3 \cdot Et_2O$, toluene or CH_2Cl_2.

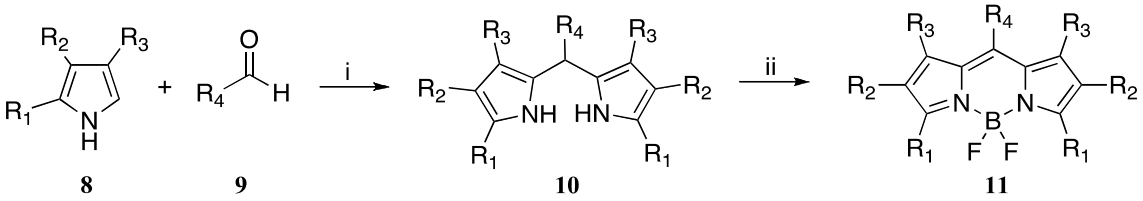
Acid anhydrides such as glutaric anhydride and succinic anhydride have been used as an alternative of acyl chlorides in the synthesis of BODIPY fluorophores.[17] BODIPY derivative **7** was generated from succinic anhydride **6** and pyrrole fragment **5**. (Schemem1-2) This chemistry is particularly useful for the preparation of BODIPY with a free carboxyl group that can be further conjugated to targeted molecules.



5 **6** **7**

Scheme 1-2: *Reagents and conditions*: i) $BF_3 \cdot Et_2O$; (ii) NEt_3, $BF_3 \cdot Et_2O$.

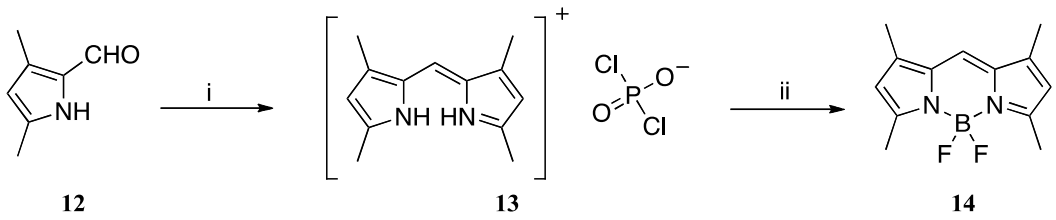
Alternatively, aldehyde **9** can be used in place of acyl chloride or anhydride as the electrophilic carbonyl substrate. Thus, dipyrromethane intermediate **10** can be formed by condensation of pyrrole **8** and aldehyde **9** in the presence of an acid. The dipyrromethane intermediate **10** needs to be further oxidized into the corresponding dipyrromethene followed by complexation with borontrifluoride in the presence of a base (Scheme 1-3). Although this synthetic strategy involves more steps, it is still commonly used owing to the stability of dipyrromethane.



8 **9** **10** **11**

Scheme 1-3: *Reagents and conditions*: i) H^+, CH_2Cl_2; ii) (a) 2,3-dichloro-5,6-dicyano-l,4-benzoquinone (DDQ), (b) NEt_3, $BF_3 \cdot Et_2O$.

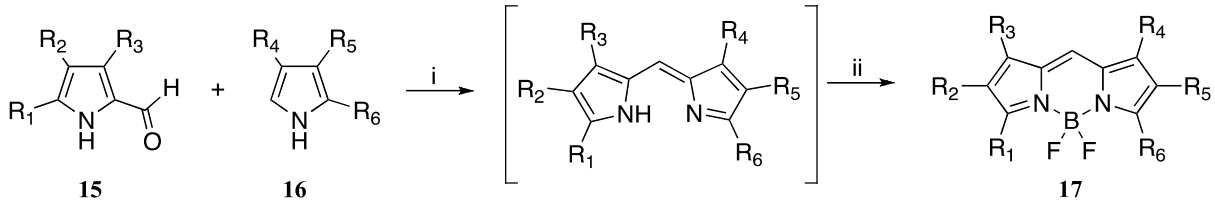
Wu's group reported a novel approach towards the synthesis of BODIPY framework (Scheme 1-4).[18] In the presence of excess phosphorus oxychloride, pyrrole-2-carbaldehyde derivatives **12** are condensed to form dipyrromethene dichlorophosphate salt intermediates **13**. Without further purification, the intermediates were treated with a base and borontrifluoride to generate the target symmetric BODIPY derivatives **14** unsubstituted at the *meso*-position in high yields.



12 13 14

Scheme 1-4: *Reagents and conditions*: i) $POCl_3$, CH_2Cl_2, room. temp., 12 h; ii) NEt_3, $BF_3{\cdot}Et_2O$, room. temp., 12 h.

Routes outlined above are convenient strategies to obtain symmetrically substituted BODIPY dyes. Asymmetric BODIPY fluorophore **17** can usually be obtained by the MacDonald coupling approach (Scheme 1-5), via the condensation reaction of a carbonyl-containing pyrrole **15** with pyrrole **16** that is unsubstituted at the α-position. Yields for the MacDonald reaction are generally high. However, dramatically reduced yields are often observed when the α-free pyrroles are electron-deficient.[19] In the latter case, the favored reaction is the self-condensation of pyrrole-2-carbaldehyde derivatives, and undesired symmetric BODIPYs are always isolated as the major products.[19] Overall the main advantage of this strategy is the possibility to functionalize BODIPYs on pyrrole carbocycles side chains, which enables them to be further derivatized or attached to other macromoleculars.



15 16 17

Scheme 1-5: *Reagents and conditions*: i) H^+, CH_2Cl_2; ii) NEt_3, $BF_3{\cdot}Et_2O$.

1.4 Fluorescent properties of BODIPY

Fluorophores have several important characteristics such as fluorescence lifetime, quantum yield, maximum absorption and emission wavelengths, Stoke's shift and molar extinction coefficient. In general these properties are readily influenced by the structures of fluorophores, some of which are described below.

1.4.1 General structural features of fluorophores

Fluorophores typically contain rigid frame structures that have combined aromatic groups, planar or cyclic structures with elongated conjugated π systems. The fluorescence properties are influenced by several factors, such as π-electron conjugation, structural rigidity, and the type and position of substituents on the molecules.

1). Increasing π-electron conjugation normally leads to enhanced fluorescent emission and red-shifts in excitation and emission wavelengths. By modifying the conjugation system, possible decrease or enhancement of quantum yield can be observed.

2). Introducing steric constraints to the molecular structure normally enhances the fluorescence quantum yield. The increased rigidity, induced by conformational restriction, reduces the rotation and distortion of the molecule, which accounts for increases in quantum yields.[20]

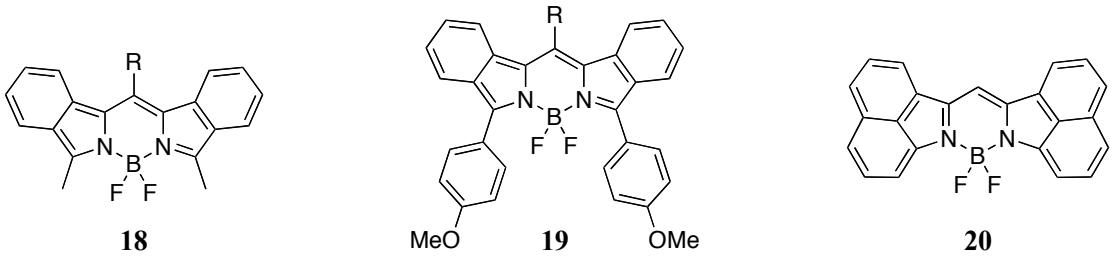
1.4.2 Structural modification of BODIPY

BODIPY fluorophores, with a rigid core structure, quite commonly possess extraordinary photochemical and photophysical properties. A wide range of structural modifications, tailoring the fluorescent properties, have been performed on the BODIPY fluorophore. Selected BODIPY analogues with extended conjugation systems and with modifications on

rigidity and the boron centre will be discussed herein.

1.4.2.1 Extension on π-conjugation systems

Some researchers successfully extended the π-conjugation systems to red-shift the excitation and emission wavelengths of BODIPY dyes.[21-24] Several strategies can be used to expand the conjugation, including synthesizing the di(isoindole)methane dyes [21a, 21b] or attaching ethynylphenyl, styryl, vinyl, aromatic ring substitutions to the framework.[22] A handful of publications have been reported to utilize Retro-Diels Alder reaction to access the highly conjugated BODIPY analogues.[21a, 23] Mitasuo *et. al*.[21a] reported that the isoindole-BODIPY dyes **18** (Figure 1-3) could be generated *via* a retro-Diels–Alder reaction from bicyclo[2.2.2]octadiene fused BODIPY precursors. A few years later, the same group prepared a new class of π-expanded BODIPYs from the precursor dyes fused with benzene, acenaphthylene, and benzofluoranthene by the same strategy.[23] Other than the retro-Diels-Alder reaction, some other routes have been utilized to prepare isoindole-based BODIPY derivatives that exhibit fluorescence absorptions and emissions in visible or near-infrared region. As reported, substituted 2-acylacetophenone was condensed with ammonia to give the dibenzopyrromethene and the corresponding product was then treated with boron trifluoride diethyl etherate to generate the targeted BODIPY **19** with 3,4,3',4'-dibenzopyrrometheneboron difluoride core.[24a] Another interesting example with extended conjugation system, compound **20**, can be obtained by condensation of 2-methylthiobenz-[c,d]indolium iodide and 2-methylbenz[c,d]indolium iodide, respectively, followed by addition of a base and complexation with boron trifluoride diethyl etherate.[24b] The corresponding product has fixed planar structure, exhibiting a sharp absorption band at 618 nm and emission band at 625 nm.



18

EtOH, Φ 0.28

λ_{max}^{ab} 603 nm

λ_{max}^{em} 609 nm

19

$CHCl_3$, Φ 0.71

λ_{max}^{ab} 646 nm

λ_{max}^{em} 671 nm

20

DMSO, Φ 0.52

λ_{max}^{ab} 618 nm

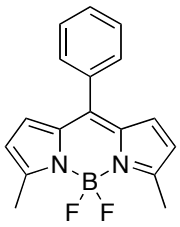
λ_{max}^{em} 625 nm

Figure 1-3. BODIPY dyes with extended conjugations

Interestingly, aryl substitution at the *meso*-position does not substantially alter the maximal absorption and emission profile[11] while substitution at the α-positions of BODIPY can effectively shift the absorption and emission wavelengths.[22]

1.4.2.2 Restricted systems

As mentioned above, arylation at the *meso*-position has no specific effect on the absorption and emission wavelength while the quantum yield of the *meso*-phenyl compound suffered a significant reduction when free rotation of the aryl group is allowed.[11] However, high fluorescence quantum yields can be restored by introducing methyl groups to the β-positions of the BODIPY framework (Figure 1-4: compare **21** with **22**). In fact, blocking the 1,7-positions can inhibit the free rotation of the phenyl group, which consequently reduces loss of energy from the excited states via non-irradiative molecular motions. Consistent with this, greatly enhanced quantum yields also can be observed through introducing *ortho*-substituents on the phenyl ring **23**, and similar explanations have been invoked. (Figure 1-4: compare **21** with **23**) Consequently, increasing the rigidity of the BODIPY structure is an efficient strategy to obtain the high fluorescence intensity.

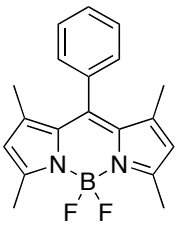


21

MeOH, Φ 0.19

λ_{max}^{ab} 508 nm

λ_{max}^{em} 521 nm

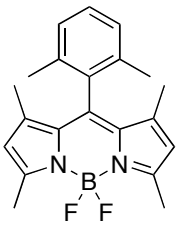


22

MeOH, Φ 0.65

λ_{max}^{ab} 498 nm

λ_{max}^{em} 508 nm



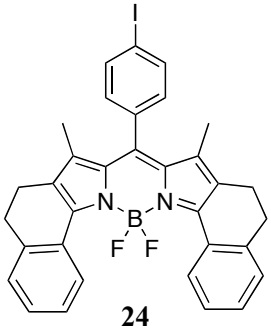
23

MeOH, Φ 0.96

λ_{max}^{ab} 500 nm

λ_{max}^{em} 510 nm

Figure 1-4 Effects of *meso*-aryl substitution of the fluorescence of BODIPY. Reduced quantum yields are observed in unsubstituted *meso*-aryl BODIPYs

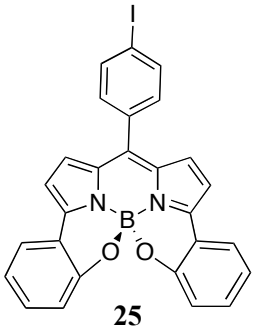


24

MeOH, Φ 0.72

λ_{max}^{ab} 619 nm

λ_{max}^{em} 629 nm



25

$CHCl_3$, Φ 0.41

λ_{max}^{ab} 630 nm

λ_{max}^{em} 654 nm

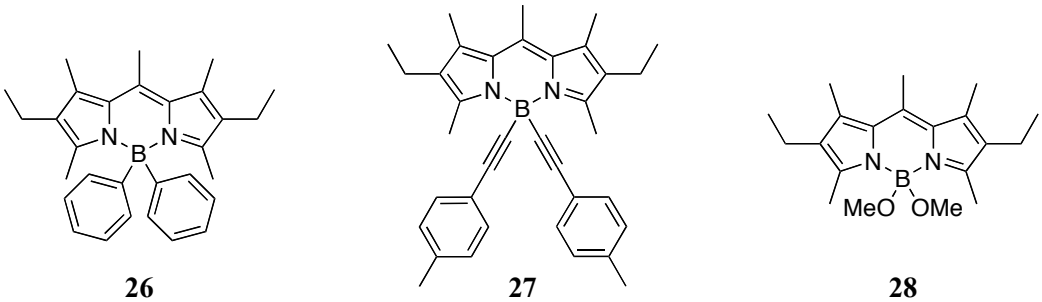
Figure 1-5 BODIPY dyes with rigid structure

Kevin Burgess' lab[25] prepared a series of BODIPY dyes with more rigid conformations in which the aryl substituents and 2, 6-position of the BODIPYs have been linked through a heteroatom or ethylene bridge precluding free rotation of the substituted-benzene molecular fragments. These dyes exhibit visible excitation and emission wavelengths [λ_{max}^{ab} (620-660 nm), λ_{max}^{em} (630-680 nm)] and high fluorescent quantum yields (up to 0.72 for **24**). Another novel modification on the rigidity has been shown by the same group. The aryl-substituted dye is constrained through an ether bond linkage between the boron center and aryl groups.

The constrained dye **25** [26] exhibits a sharper, red-shifted fluorescence emission that is more intense than the precursor analogue with aryl groups rotating freely.

1.4.2.3 Modification at the boron center

Replacement of the two fluorine atoms in BODIPYs provide a novel strategy to generate a new family of BODIPYs, which is considered as an alternative way to modify some optical properties of BODIPYs (Figure 1-6). Aryl displacements on the boron-fluorine bond (compound **26**) entity can efficiently extend the conjugation system,[27] which was first reported in Murase's patent.[27a] The novel approach was further exploited by Ulrich and his co-workers in developing BODIPY derivatives with aryl, ethynylaryl (compound **27**), and ethynyl subunits in place of fluorine atoms.[27b, c] Compared with the F-BODIPY, these aryl-BODIPY dyes show red-shifted excitation and emission wavelengths with minimal loss of fluorescence. Afterwards, more work has been reported on BODIPY analogues where fluorine is replaced with alkyl,[28] aryloxy, alkoxy (compound **28**) and hydroxyl groups.[29] Conducting substitution reactions at the boron center has dramatically increased the versatility of BODIPY type fluorophores.



26

MeOH, Φ 0.91
λ_{max}^{ab} 513 nm
λ_{max}^{em} 548 nm

27

MeOH, Φ 0.95
λ_{max}^{ab} 516 nm
λ_{max}^{em} 537 nm

28

MeOH, Φ 0.49
λ_{max}^{ab} 498 nm
λ_{max}^{em} 509 nm

Figure 1-6 Structures and fluorescent properties of BODIPY derivatives with variation on boron substituent

1.4.2.4 Commercially available BODIPY dyes covering the visible spectrum

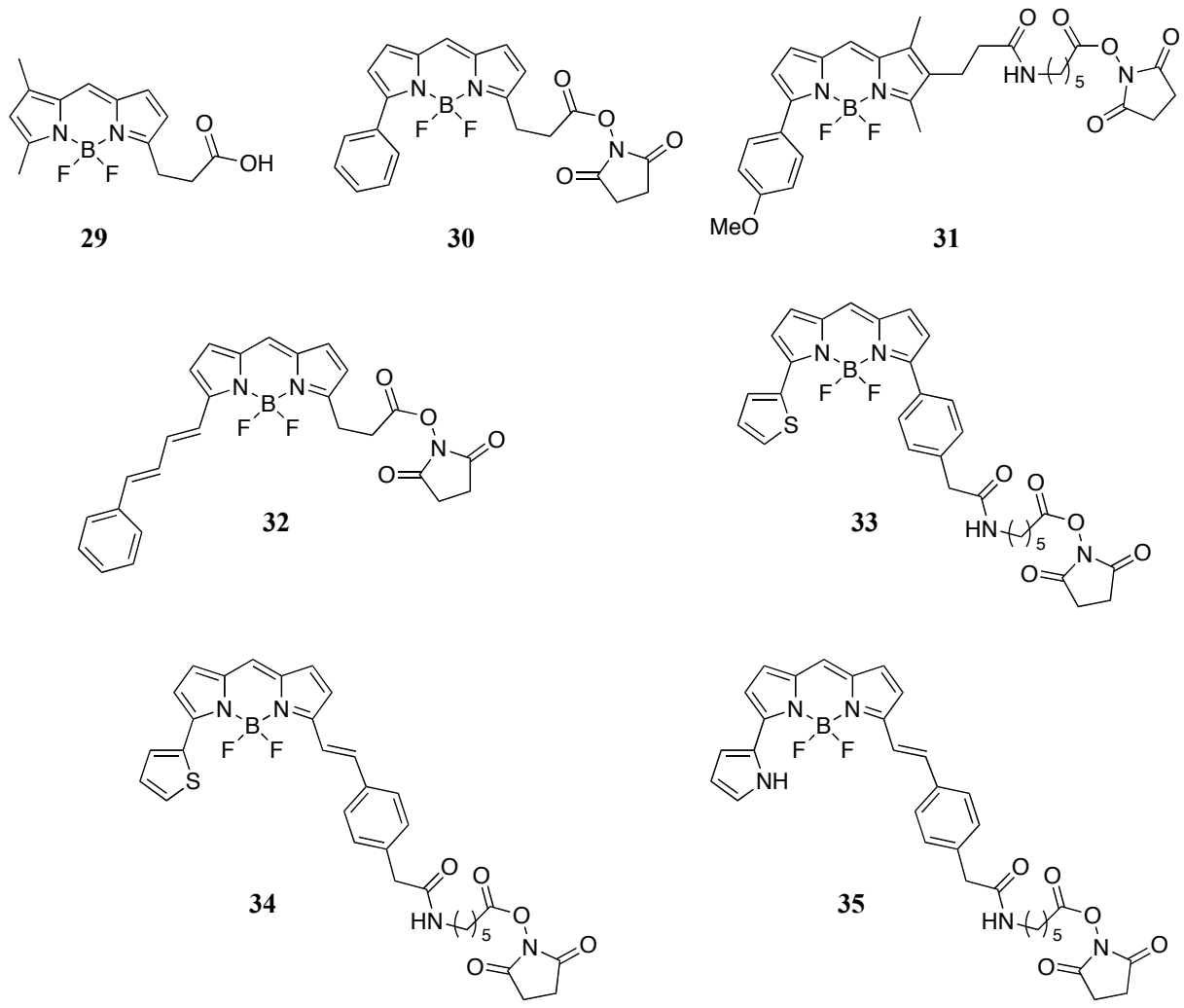
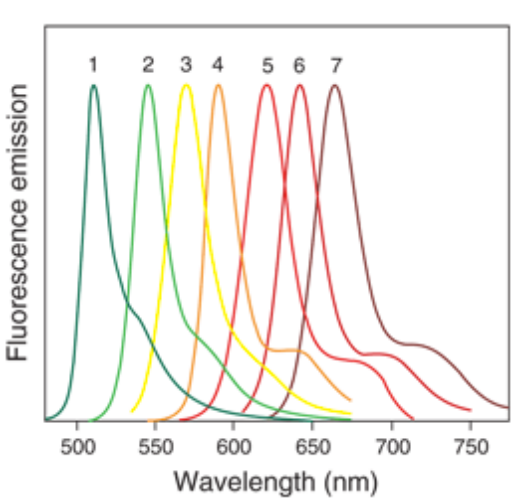


Figure 1-7 Chemical strutures of commercially available BODIPY fluorophores spanning the visible spectrum.



	Structure	Analogue
1	29	BODIPY FL
2	30	BODIPY R6G
3	31	BODIPY TMR
4	32	BODIPY 581/591
5	33	BODIPY TR
6	34	BODIPY 630/650
7	35	BODIPY 650/665

Figure 1-8 Normalized fluorescence emission spectra of BODIPY derivatives in methanol.[12]

α-Substituted conjugated systems can effectively shift the maximal emission wavelength. Compounds **29-35** (Figure 1-7), covering the majority of the visible spectrum (Figure 1-8), are commercially available BODIPY from Invitrogen.

1.5 Applications of BODIPY dye

Synthesis of BODIPY fluorophores was first reported in 1968.[6] However, their applications as fluorescent sensors or labels only became common practice some twenty years later. Some applications of the BODIPY fluorophores are summarized below.

1.5.1 Chemosensor

Fluorescent sensing is a very useful approach in the detection and quantification of specific molecules and in imaging, and thus fluorescent sensors play a critical role in research geared towards the understanding of chemical and biological systems. To this end, BODIPY dyes have been synthesized as candidates for selective sensor of cations,[34-44] anions,[46-49, 51]

redox active molecules,[52-54, 56, 59-61] pH probes[64, 65] and probes for biomolecules such as peptides, proteins, lipids and nucleic acids.[66-69]

1.5.1.1 Metal ion sensors

Metal ions are involved in diverse biological processes.[30] Heavy metal ions, such as lead, cadmium and mercury ions, can cause adverse health effects upon exposure.[31] Besides, these heavy metal ions can accumulate in the environment, leading to contamination in food and water.[32] Fluorescence is a simple and inexpensive method to detect and quantify heavy metal ions in real-time monitoring of environmental, biological, and industrial samples.[33] To this end, sensitive and selective BODIPY sensors (Figure 1-9) have been developed for fluorometric analysis for ions such as Pb^{2+},[34] Ag^{+},[35] Hg^{2+}, [35 b, 36] and Cd^{+}.[37]

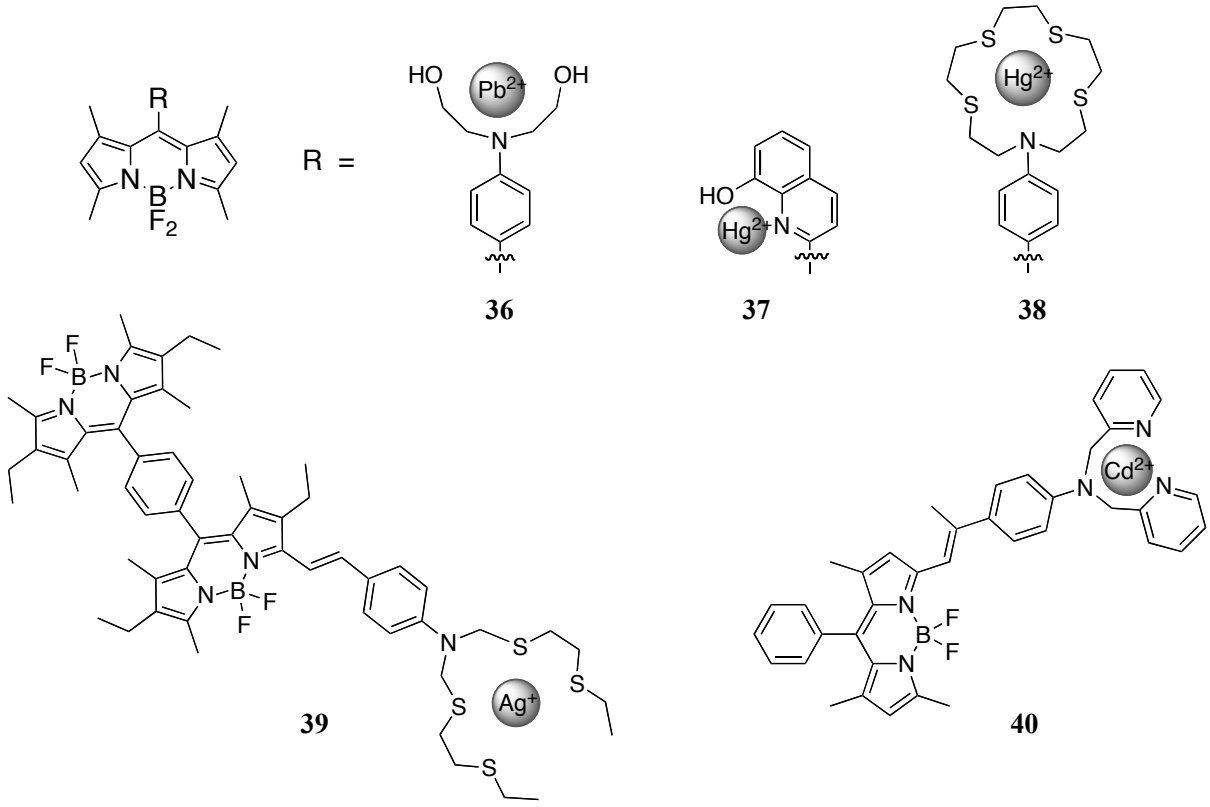


Figure 1-9 Structures of some BODIPY heavy metal ion sensors

Compound **36**[34 c] was designed as an "off-on" mercury sensor, exhibiting a selective

chelation enhanced fluorescence (CHEF) effects with Pb^{2+}. The dramatic enhancement of fluorescence was owing to the blocking of the photoinduced electron transfer (PET) process. Similarly, the thio-aza-crown chemosensor **38** [36d] was found to selectively detect Hg^{2+} by an "off-on" type effect. Selective Hg^{2+} ion chemosensor **37**, however, exhibits pronounced Hg^{2+} selective on-off-type fluoroionophoric properties. Thus, the fluorescence of compound **37** can be efficiently quenched by more than 98% with addition of five molar equivalents of Hg^{2+} ions.[36c] Binding of **37** to Hg^{2+} ions is also accompanied by a color change from light amber to red that is visible to the naked eye. It is noted that most chemosensors display changes in fluorescent intensity, while only very few of them can exhibit spectral shifts in either excitation or emission. Binding of **39** to Ag^{+} results in a concentration-dependent red-shift in emission from 630 to 670 nm, accompanied by fluorescent enhancement upon binding.[35a]

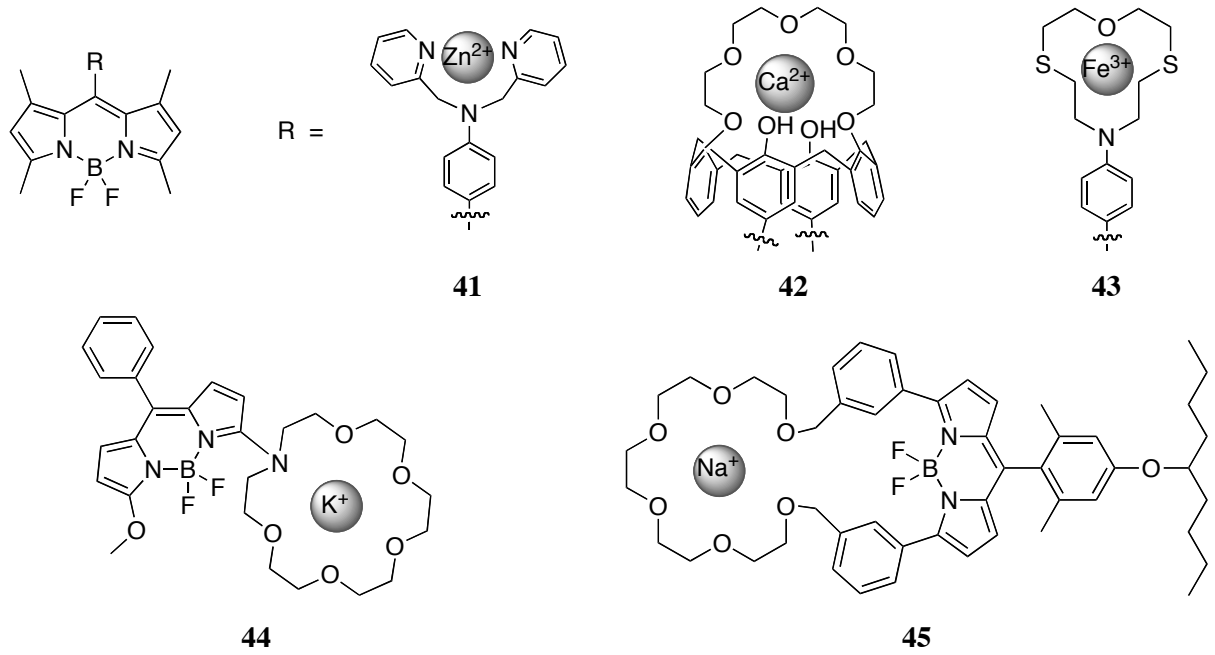


Figure 1-10 Structures of some BODIPY Na^{+}, K^{+}, Ca^{2+}, Zn^{2+}, Fe^{3+} metal ion sensors

Other metal ions such as alkali, alkaline earth metal and other transition metal ions are abundant in living organisms for different functions.[38] For instance, alkali metal ions, such

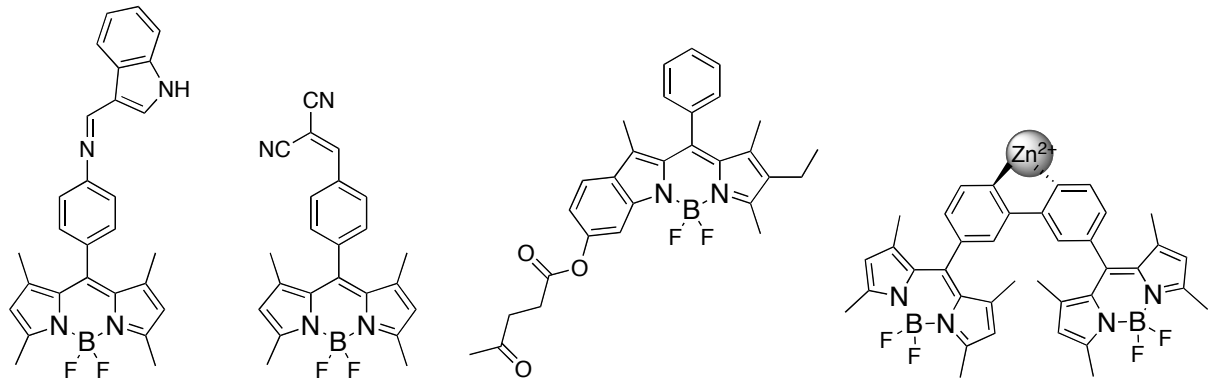
as Na^+ and K^+, are crucial in establishing and regulating cellular membrane potentials. Ca^{2+} ions play an important role in living organisms, where they can function as regulators for many enzymes and proteins. Besides, Zn^{2+}, Ca^{2+} or Fe^{3+} ions have irreplaceable effects on biological structures and activities. Thus, a variety of fluorescent indicators for ions, such as Na^+,[39] K^+,[40] Ca^{2+},[41] Cu^{2+},[34c, 42] Zn^{2+},[43] or Fe^{3+}[44] have been designed (Figure 1-10). Most of these fluorophore-receptor systems are based on either photoinduced electron transfer (PET) or internal charge transfer (ICT) processes. In sensor **41**, PET is inhibited upon binding to Zn^{2+}, leading to increase in fluorescence intensity.[43a] Ionophore **42** shows a selective 'on-off' type quenching in response to Ca^{2+} due to the PET effect. Generally PET-based sensors show fluorescence intensity changes upon binding to non-metal ligands without significant shifts in the absorption and emission spectra.[41] Binding of metal ions, however, often leads to spectral shifts in addition to changes in emission intensity. For example, fluoroionophore **45** exhibits a red shift in the absorption maximum by 12 nm in the presence of Na^+ ions, which is likely due to conformational changes, induced by the formation of **45**-Na^+ complex.[39a] Similarly, a considerable conformational change of K^+ sensor **44** can be observed upon K^+ ion binding, leading to a large (em and ex) wavelength shift.[40b] Some fluorescent indicators show changes in fluorescent properties through the formation of new compounds. Ferric ion sensor **43**,[44a] for example, displays high sensitivity and selectivity for Fe^{3+} both in aqueous solution and living cells, showing turn-on fluorescence upon addition of ferric salts. It was presumed that this effect was based on quenching of PET affected by the hydroxylamine oxidation reaction.

1.5.1.2 Anion sensors

Much like cations, anions also play crucial roles in diverse essential biological

processes.[45] To this end, numerous fluorescent sensors have been developed for anions. Some BODIPY-derived anion indicators for F^-,[46] CN^-,[47] HSO_4^-,[48] SO_3^{2-},[50] and HPO_4^{2-} [51] are described in this section (Figure 1-11).

Compound **46**[48] was synthesized as a highly selective 'off–on' fluorescent sensor for hydrogen sulfate anion (HSO_4^-) in neat and aqueous acetonitrile. In neat CH_3CN, the 'switch on' process was due to the suppression of PET by forming hydrogen binding between the HSO_4^- and the -C=N- of the receptor **46**. In aqueous acetonitrile, fluorescent changes can be explained by the protonation of N atom (-N=C), leading to the inhibition of the PET process.



46 **47** **48** **49**

Figure 1-11 Structures of some BODIPY-based anion sensors

Cyanide, an extremely toxic species, is used in various industrial processes. Some BODIPY derivatives have been synthesized as excellent cyanide anion indicators (Figure 1-11). For instance, compound **47**[47b] displays fluorescence turn-on in the presence of cyanide. The delocalization of the lowest unoccupied molecular orbital (LUMO) over the phenyl and dicyano-vinyl groups results in the photo-induced intramolecular charge transfer (ICT) from BODIPY to dicyano-vinyl groups. The π-conjugation between phenyl and dicyano-vinyl groups can be interrupted by the attacking of cyanide on the olefinic carbon, which leads to

fluorescence switch on.

Sulfites are commonly used as preservatives or enhancers in food and beverage industry. However sulfite may cause asthma attacks or other allergic reactions in hypersensitive persons.[49] A few BODIPY-based fluorescent sulfite sensors have been prepared for the identification and quantification of sulfites. For instance, compound **48**,[50] an indole-BODIPY based sulfite dictator, shows a ratiometric and colorimetric response to sulfite ions through an irreversible chemical transformation. The levulinyl protected BODIPY could be easily and selectively deprotected by sulfites. As a result, the corresponding BODIPY derivative displays a red shift in absorption and a dramatic color change from orange to blue.

Another example of anion sensor is the 1:1 Zn^{2+} complex with bipyridyl ligand **49** which is substituted with two BODIPY moieties. This system was found to be highly sensitive to phosphate,[51] leading to fluorescence quenching.

1.5.1.3 Sensors of reactive oxygen species (ROS) and reactive nitrogen species (RNS)

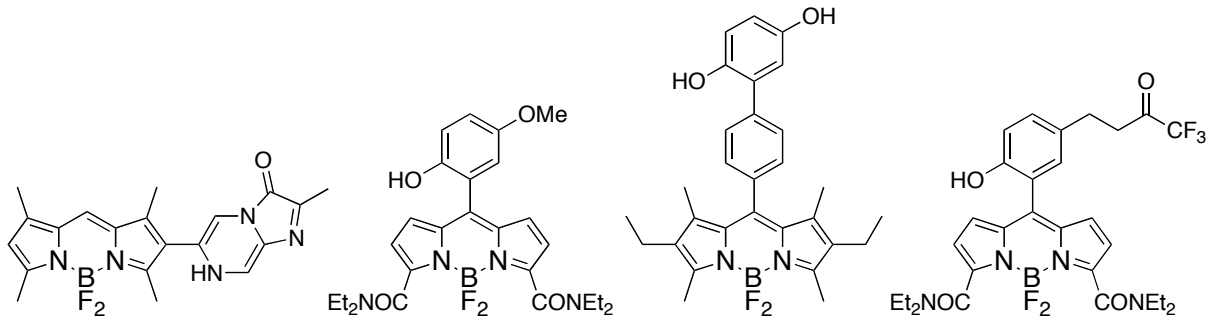
ROS and RNS include diverse species such as peroxynitrite ($ONOO^-$), peroxyl radical ($ROO^\bullet$), superoxide radical anion ($O_2^{\bullet -}$), hydroperoxyl ($HOO^\bullet$), and nitric oxide (NO) and molecules that readily give rise to radical species, such as hypocholrous acid (HOCl), hydrogen peroxide (H_2O_2) and nitroxyl (HNO). ROS are natural byproducts of the normal metabolism of oxygen.[52, 53] Notably, these species play crucial roles in cell signaling and homeostasis.[53, 54] ROS level has close relevance with cell metabolism, which has been well documented.[52] Hence, the detection of ROS is of great importance. Fluorescence detection is a simple and effective protocol, and in general fluorescent ROS/RNS indicators are dosimeters due to the irreversible reaction between probes and ROS/RNS species.

In 2009, Sekiya reported a novel luciferin-based BODIPY probe **50** (Figure 1-12) for the

detection of reactive oxygen species.[55] The chemiluminescent probe was oxidized by ROS and exhibit emission at 545 nm under neutral pH. Among the various ROSs tested, the BODIPY analogue **50** displayed the highest relative chemiluminescence intensity (RCI) in the presence of $O_2^{\bullet -}$.

BODIPY dye **51** is another example of a ROS sensor. This fluorophore is capable of detecting hypochlorite (OCl^-) anion on the basis of a specific reaction with *p*-methoxyphenol.[56] Upon formation of benzoquinone, the HOMO energy level of the *meso*-substituents is lowered, which prohibits the PET process and as a consequence fluorescence is restored. The probe was observed to successfully detect the formation of HOCl in an enzymatic system and in living macrophage cells upon stimulation.

Last but not least, Andrew *et. al.* reported a BODIPY based peroxide probe that has redox active quinone units appended at the *meso*-position.[57] The conversion of hydroquinone units to quinone products resulted in the observation of intense fluorescence, especially with a phenylene spacer **52**.

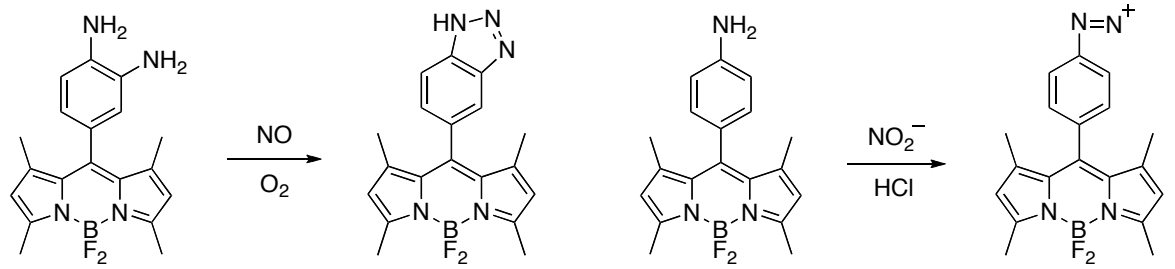


50 **51** **52** **53**

Figure 1-12 Structures of some BODIPY-based ROS sensors.

RNS, like ROS, are signaling molecules required for normal cellular functions.[58] Peroxynitrite is a short-lived ROS and RNS, formed *in vivo* from nitric oxide and superoxide in a 1:1 ratio. Yang's group developed a novel BODIPY dye **53**[59] that was highly sensitive

and selective for the detection of peroxynitrite based on a PET mechanism. A specific reaction occurs between peroxynitrite and phenoxyphenyl-derived ketone on nonfluorescent probe **53,** which forms a highly fluorescent BODIPY derivative. Furthermore the probe was proved to successfully detect perxoynitrite generated in murine macrophage cells activated by phorbol 12-myristate 13-acetate, interferon-γ and lipopolysaccharide.



54 DMSO, Φ 0.001 λ^{ab}_{max} 496 nm λ^{em}_{max} 505 nm

55 DMSO, Φ 0.40 λ^{ab}_{max} 498 nm λ^{em}_{max} 507 nm

56

57

Figure 1-13 Structures of BODIPY-based nitric oxide and nitrite sensors

Nitric oxide (NO), as a typical RNS/ROS second messenger, is involved in the regulation of numerous physiological and biological processes.[60] However, *in situ* detection of NO radicals has been greatly limited due to the short life and low physiological concentrations of NO radicals. The most common strategy for fluorescent NO sensing is to employ *o*-phenylenediamine, a NO-responsive motif.[61, 62d] Compound **54**[62a] is BODIPY-derived NO probe. Upon reaction with nitric oxide in the presence of oxygen, the BODIPY dye **54** (Φ = 0.002) exhibited great enhancement in quantum yield (Φ = 0.74) with the formation of the corresponding benzotriazole, which can be explained by the reductive PET mechanism. Several other groups have reported additional BODIPY probes for NO.[62b-d]

Another kind of RNS species, nitrites, plays a pivotal role in environmental, food,

industrial and physiological systems.[63] Due to the importance of nitrites, there has been significant interest in trace nitrite determination in recent years. Li *et. al*.[64] prepared BODIPY reagent **56**, 1,3,5,7-tetramethyl-8-(4'-aminophenyl)-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacence (TMABODIPY) to determine trace nitrite. Upon the irreversible reaction of nitrite with TMABODIPY first in acidic solution and then in alkaline solution, BODIPY diazonate derivative **57** was formed as a stable and extremely fluorescent product (Figure 1-13).

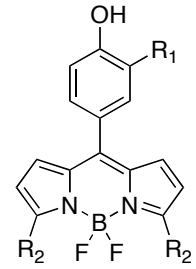
1.5.1.4 pH sensors

Numerous research has been focused on the design and construction of pH indicators inside living cells due to the importance of intracellular pH.[65, 67a] It is worth noting that most valuable probes are responsive to near-neutral, cytosolic pH around 6.8-7.4.[66]

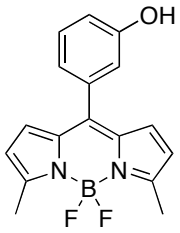
To date, no functional BODIPY-derived probes for cytosolic conditions have been shown, however, various BODIPY-based fluorescent sensors for pH detection in organic, aqueous, and mixed media have been reported.[67] Figure 1-14 shows the structures of some BODIPY-based pH sensor. Baruah's group reported seven BODIPY dyes **58**[67d] bearing phenolic or naphtholic subunits with p*K*a values between 7.5 and 9.3. In aqueous solution, compounds **58 a-c** and **e-f** were demonstrated as fluorescent pH probes whose fluorescence emission and excitation intensities are enhanced upon decrease of pH. Further study was not feasible to compound **58d** owing to its poor solubility in aqueous solution.

Sensors that are sensitive to more acidic or more basic biological environments such as lysosomes have also been under development. Lysosomes, distinct from other cellular organelles, have a low pH (5.0–6.0). Thus, lysosomal labeling should be activated only in acidic conditions while remain nonfluorescent in neutral conditions, or *vice versa*. Branchaud

and co-workers described BODIPY-based, pH-activated probes **60** with *p*Ka values ranging from 3.2 to 5.2.[68a] These pH probes displayed intense fluorescence at pH 4, and the fluorescence intensity is reduced at neutral pH. At pH over 7, the fluorescence of these pH probes was completely quenched. The dyes can be used for selective lysosome labeling as well as in monitoring lysosomal pH changes during physiological and pathological processes. Similarly, compounds **61** were designed as pH-responsive fluorescent probes for selective imaging of cancer cells *in vivo*.[68b]



a R^1,R^2= H
b R^1=H, R^2 = CH_3
c R^1=H, R^2 = Cl
d R^1= H, R^2= *p*-MeOPh
e R^1=Cl, R^2 = CH_3



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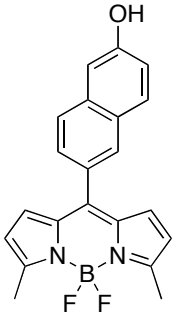

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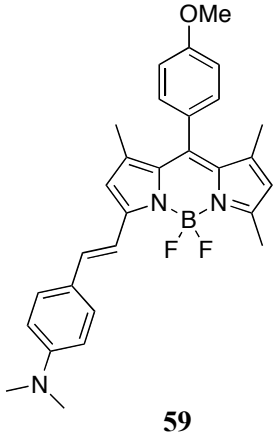

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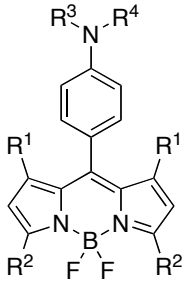
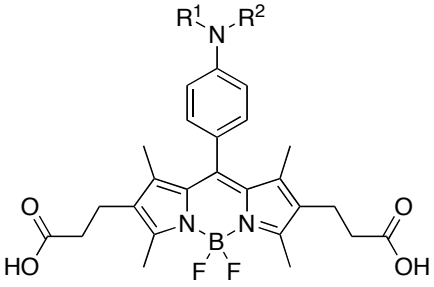


60

a R^1,R^2, R^3,R^4 = CH_3
b R^1,R^2 = CH_3, R^3,R^4=CH_2CH_3
c R^1,R^2= CH_3, R^3 = CH_2CH_3,
R^4= $CH_2CH_2CH_2CO_2Me$
d R^1= H, R^2= $CH_2CH_2CH_2CO_2Me$
R^3,R^4 = CH_2CH_3



61

a R^1,R^2 = H
b R^1,R^2 = CH_3
c R^1= CH_2, R^2 = CH_2CH_3
d R^1,R^2 = CH_2CH_3

Figure 1-14 Structures of some BODIPY pH sensors

1.5.1.5 Indicators for biomolecules

BODIPY based sensors for biomolecles are relatively rare, however, some BODIPY analogues have been developed for the detection and quantification of biomolecules such as monosaccharides,[69] thiols,[70] proteins[71] and peptides[72].

Boronic acid and esters have been shown as important functional groups in the design of carbohydrate sensors.[69] Lakowicz *et. al*. synthesized monosaccharide indicator **62** (Figure 1-15), a BODIPY type dye functionalized with a boronic acid group.[69a] In the presence of monosaccharides (D-fructose, D-galactose and D-glucose), the BODIPY sensor exhibits changes in both absorption and emission spectra. Compound **63** shows a 24-fold fluorescence enhancement upon recognition of D-fructose.[69b] This dye was found to be highly selective for D-fructose among 24 saccharides.

Molecules that contain thiols, such as cysteine (Cys), glutathione (GSH), and homocysteine (Hcy), are related to a variety of biological processes such as redox homeostasis and metal ion binding.[73] Most thiol-sensors contain 2,4-dinitrobenzenesulfonyl (DBS) group, an established quencher in the design of thiol sensor. Compound **64**[70a] is a selective thiophenol probe, associated with a color change from red to yellow and 63-fold enhancement in fluorescence upon reaction with thiols. This sensor has also been shown to be useful in imaging and discriminating thiols in living cells. Other BODIPY containing 2,4-dinitrobenzenesulfonyl group as aliphatic thiols sensors have also been reported.[70b-d]

BODIPY dye **65** was developed as an imaging probe for site-specific labeling of peptides that contain two pairs of Arg-Cys in living cells.[71] Upon covalent coupling to peptides, a large spectral shift in emission can be observed due to disruption of the conjugation system. BODIPY **66** was a site-specific probe designed for labeling and imaging of β-amyloid (Aβ) aggregates in brain tissues.[72e] This fluorophore displayed a high affinity for synthetic Aβ

aggregates in *in vitro* binding experiments.

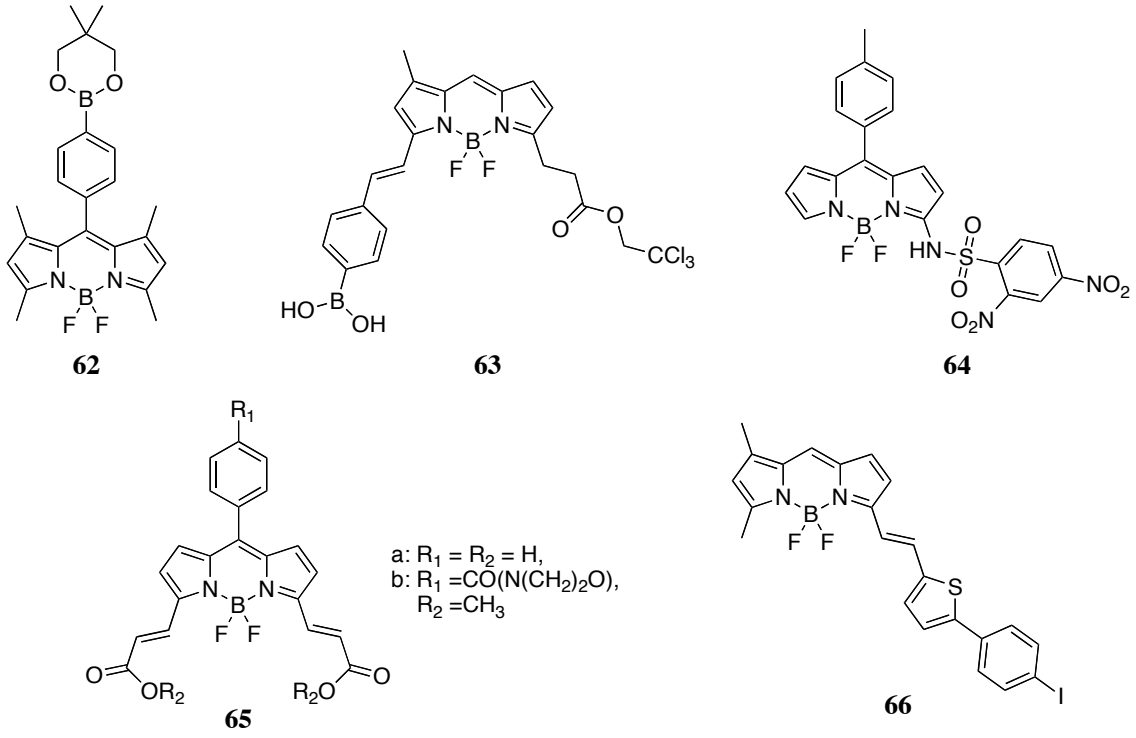


Figure 1-15 Structures of BODIPY biomolecule sensors

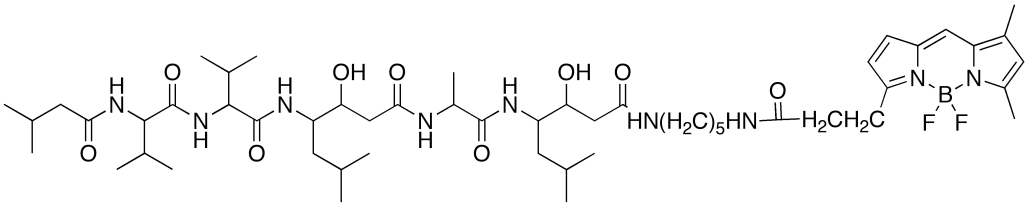
1.5.2 BODIPY dye conjugates and their applications

Recent years have witnessed a considerable and constant increase in visualization and investigation of interactions between proteins, enzymes, oligonucleotides, as well as other biomolecules in living cells. Fluorescent labeling has proved to be a powerful tool for *in vitro* detection of these interactions.

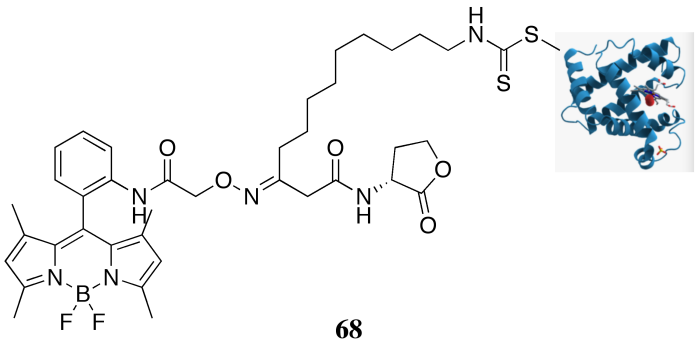
BODIPY dyes possess excellent optical and chemical properties and low toxicities,[74] making them one of the most promising candidates as fluorescent probes and labels. Thus, diverse BODIPY-based fluorescent labels have been prepared to target at peptide,[75] proteins,[76] nucleotides,[77] oligonucleotides,[78] fatty acids,[79] phospholipids,[80] polysaccharides,[81] dextrans,[82] receptor ligands[83] and small molecule drugs[84]. Some BODIPY dye conjugates are described hereafter.

1.5.2.1 BODIPY peptide, protein conjugates

Fluorescently labelled peptides and proteins can be used to locate the receptors in cells, to quantify receptors, or to determine the receptor's affinity with ligands. Due to the excellent physicochemical properties and low toxicities,[74] a few BODIPY dyes have been conjugated with peptides[75] and proteins[76]. This is well demonstrated by the BODIPY conjugated pepstatin A **67** as a cathepsin D probe (Figure 1-16). Pepstatin A is well known as an inhibitor of cathepsin A. As the pepstatin A was tagged with BODIPY, the binding property of fluorescent pepstatin A to cathepsin D can be readily characterized by fluorescent polarization measurement.



67



68

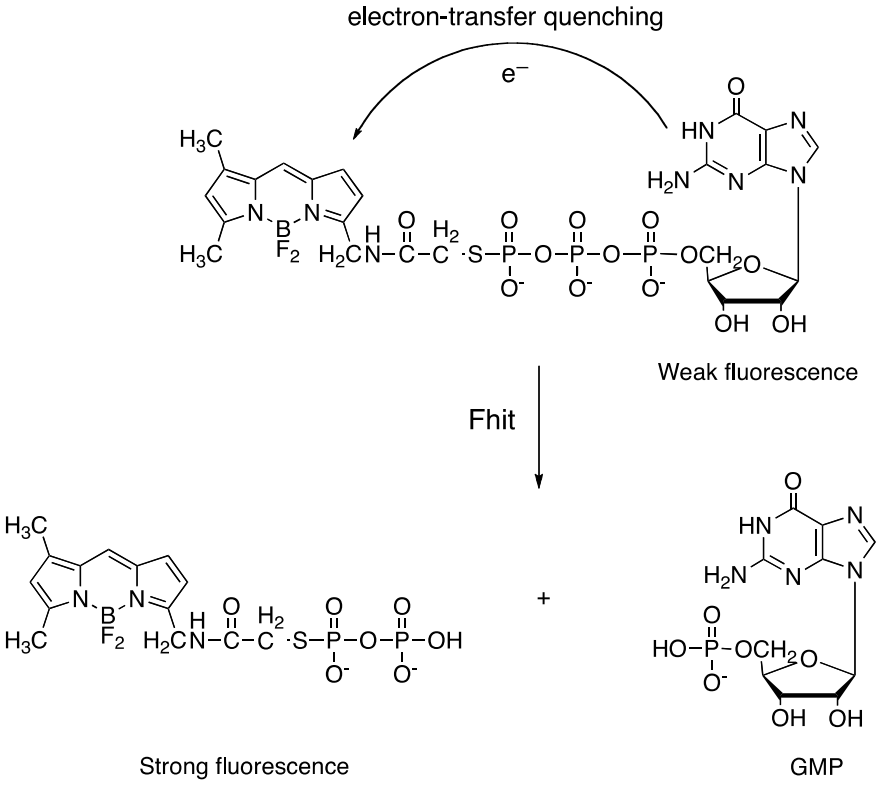
Figure 1-16 Structure of BODIPY conjugates with peptides and proteins

BODIPY-pepstatin A was also used in fixed cells and live cells as a tool for the study of the secretion and trafficking of cathepsin D.[75b] Meijler and colleagues also reported labeling of 'quorum sensing' (QS) receptors by BODIPY (as in **68**) in live cell to investigate the roles of QS receptors in cell-to-cell communication.[76b] In their approach the conjugation of

BODIPY to protein was realized through a two-step bio-orthogonal labeling strategy in which aniline was used to catalyze the oxime formation reaction between the receptor and BODIPY dye.

1.5.2.2 BODIPY Nucleotide and Oligonucleotide Conjugates

BODIPY as a small fluorophore exhibits a minimal effect on mobility in electrophoresis.[12] Thus BODIPY oligonucleotide conjugates can be used in DNA sequencing.[78a-c] BODIPY dye-labeled oligomucleotide primers have better photostability than fluorescein-labeled primers, which makes the labeled DNA more detectable in sequencing gels.[12] Additionally, BODIPY labeled single DNA strands can be used in monitoring protein synthesis by using total internal fluorescence microscopy.[78d]



Scheme 1-6. Principle of fluorescence-based detection of the diadenosine triphosphate hydrolase activity of Fhit using BODIPY GTP-γ-S thioester (G22183) as a substrate analog.

BODIPY conjugates of nucleotides can be used as structural probes of nucleotide-binding proteins,[12] enzyme substrates[77c] and tools to detect DNA damage.[77d] Invitrogen prepared BODIPY® FL GTP-γ-S thioester by linking BODIPY to GTP-γ-S (as in Scheme 1-6). The fluorescence of the fluorescent nucleotide is quenched by the PET effect, while it can be restored upon binding to G-proteins. Besides, the BODIPY FL GTP-γ-*S* thioester is an important enzyme substrate for Fhit, a member of the histidine triad superfamily of nucleotide-binding proteins. Thus, BODIPY nucleotides are useful for imaging and screening the activities of Fhit inhibitors and activators (Scheme 1-6). [12]

1.5.2.3 BODIPY lipids and other conjugates

Numerous BODIPY dye conjugates of phospholipids, drug analogs, toxins and other biomolecules (Figure 1-17) have been synthesized and applied for biological researches.

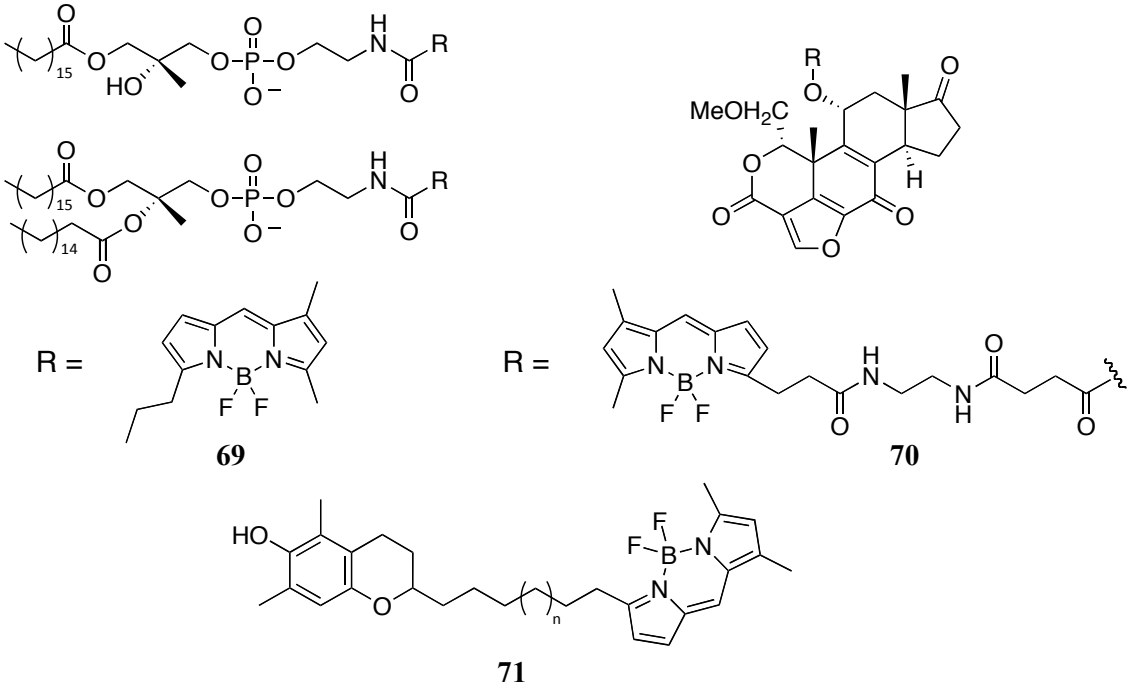


Figure 1-17 Structures of BODIPY conjugates of phospholipids, Vitamin E and drugs

BODIPY phospholipids are helpful in studying cell membrane structures and properties

such as lipid diffusion,[80a] compartmentalization of specific lipid classes in fungi and parasites,[80b] and the activity of lipids in cells.[80c] Mono- and dipalmitoyl-phosphatidylethanolamine probes **69**, both labeled in the hydrophilic head by BODIPY, were utilized to study affinity of phospholipids in various lipid bilayer systems.[80d]

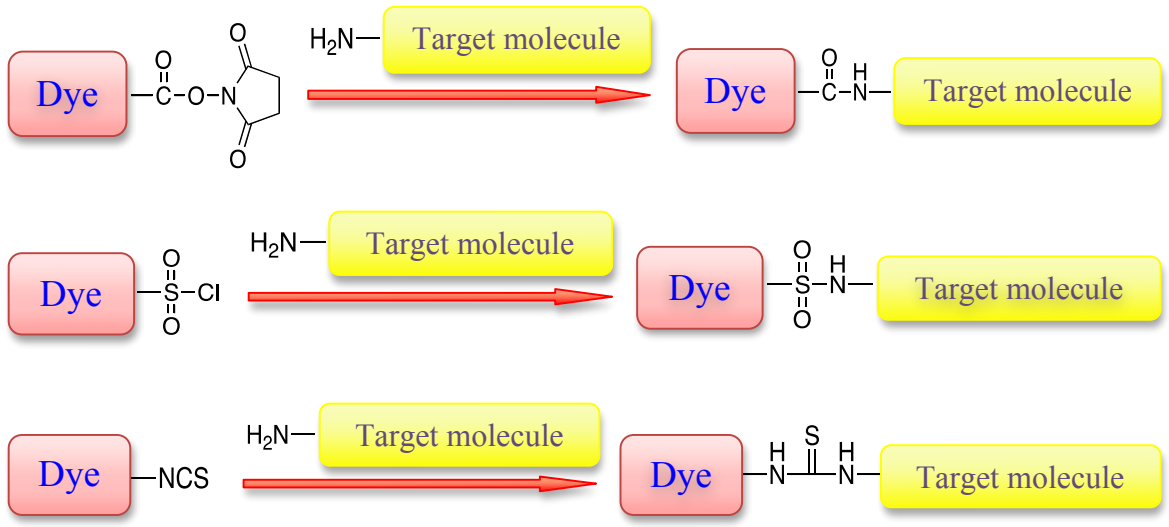
Through labeling of specific drugs, it is possible to study their *in vitro* and *in vivo* activities, such as affinities to proteins and delivery processes. Atkinson's laboratory synthesized three fluorescent analogues of α-tocopherol incorporating BODIPY fluorophore **70**. These analogues all bind specifically and reversibly to α-tocopherol transfer protein (α-TTP) with high affinity and thus can serve as fluorescent probes in the study of localization and intracellular transport of vitamin E.[84b] Another BODIPY conjugate with wortmannin **71** was prepared by Cimprich and co-workers as a cell-permeable probe to study kinases in cells.[84d]

1.6 Activated BODIPY fluorophores for labeling

In the last two decades, various fluorophores have been synthesized with different functional groups to generate fluorescent conjugates with proteins, nucleotides, dextrans as well as some phospholipids. Two major classes of activated BODIPY fluorophores are commonly used in labeling, amine- and thiol-reactive BODIPY.[12]

1.6.1 Amine-reactive BODIPY

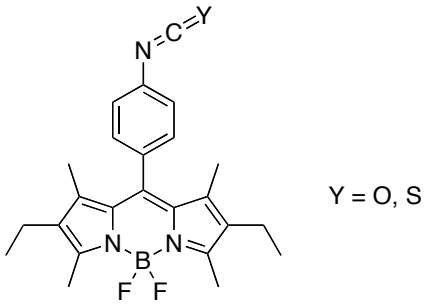
These amine-reactive fluorophores incorporate functional groups such as succinimidyl esters, sulfonyl chlorides and isothiocyanates that react readily with amines (Scheme1-7).



Scheme 1-7 Amine reactive fluorophores: a) succinimidyl esters; b) fluorescent dye sulfonyl chlorides; c) fluorescent dye isothiocyanates.

To date, succinimidyl ester derived BODIPY dyes are the most commonly used amine reactive BODIPY fluorophores. Succinimidyl esters are generally stable if kept desiccated, and upon reaction with amines, stable amide bonds are formed. Sulfonyl chlorides are reactive but the corresponding conjugates are much less stable than amides. Isothiocyanates are stable reagents in solution and form reasonably stable thioureas upon reaction with amines.

Invitrogen supplies a selection of amine-reactive BODIPY dyes. These include succinimidyl esters of several BODIPY propionic and pentanoic acids (Figure 1-4). BODIPY sulfonyl chlorides and isothiocyanates are not commercially available yet, however, recent research has demonstrated the synthesis of isothiocyanate BODIPY derivatives. Ziessel and co-workers[85] prepared BODIPY dyes **72** (Figure 1-18) substituted with isocyanato- or isothiocyanato group on phenyl that is derived at the *meso*-position. The utility of these analogues in labeling remains to be explored.



72

Figure 1-18 Structure of isocyanate, isothisocyanate BODIPY analogs

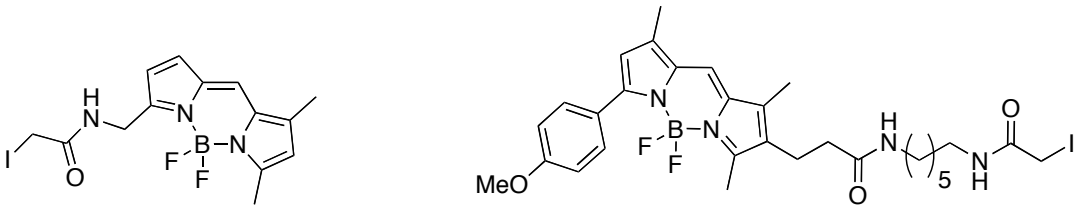
1.6.2 Thiol-reactive BODIPY

Thiol-reactive reagents are mainly based on the iodoacetamides, malemides, benzylic halides and bromomethylketones, which generate stable thioether products upon reaction by alkylation of thiols.

a) Iodoacetamides

Iodoacetamides readily react with all thiols found in peptides, proteins and thiolated polynucleotides and form corresponding thioethers.[12]

The iodoacetamide dyes BODIPY TMR cadaverine IA **73** and BODIPY Fl C1-IA **74** (Figure 1-19) were developed by Wiktorowicz's group as fluorescent labeling reagents targeting cysteine residues. These dyes show high specificity for cysteine, while little or no non-specific labeling can be observed at very low thiol : dye ratios.[86] Besides, the influences of these dyes on isoelectric points (pIs) of standard proteins are minimal. The covalent linkages formed from these reagents were also shown to be compatible with commonly available imaging equipment and in-gel digestion identification by peptide mass fingerprinting.[86]

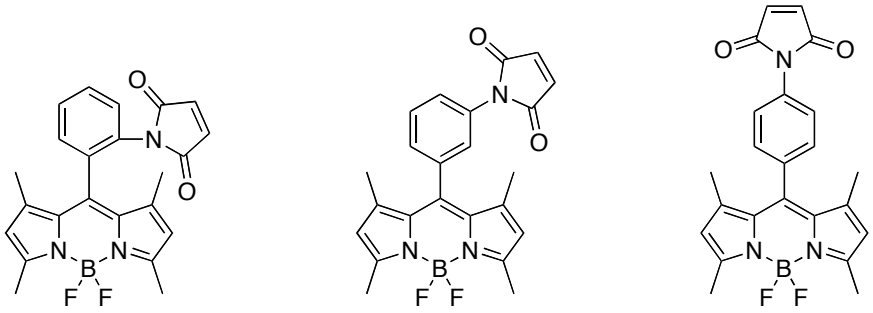


73 **74**

Figure 1-19 Structures of iodoacetamides BODIPY derivatives

b) Maleimides

Maleimides react with thiol through a 1,4-addition to generate thioethers. The fluorescent and chromophoric analogs of *N*-ethylmaleimide (NEM) are applied similarly as iodoacetamides except the reactions with methionine, histidine or tyrosine. A higher pH is required for the reaction of maleimides with amines than with thiols.[12]



Φ= 0.002 **Φ**= 0.37 **Φ**= 0.54

75a **75b** **75c**

Figure 1-20 Structures of maleimides BODIPY derivatives

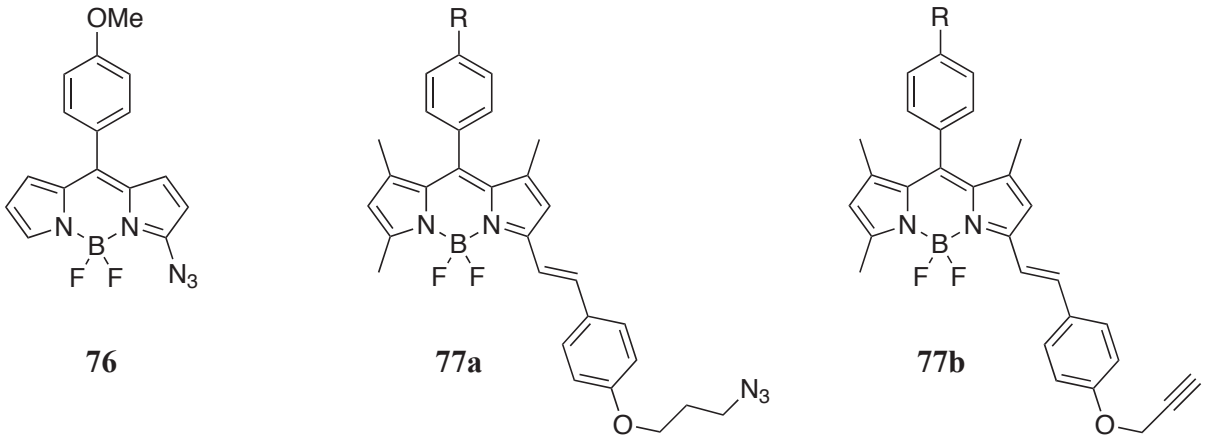
To date, examples of BODIPY maleimide derivatives have been limited. Nagano's group reported *ortho*-, *meta*- and *para*-substituted maleimide derivatives of BODIPY **75** analogs (Figure 1-20). Due to the donor-excited photo-induced electron transfer (d-PET) effect from BODIPY to maleimide, the fluorescence of *ortho*-substituted maleimide BODIPY derivatives was strongly quenched. It was observed that the fluorescence intensity was enhanced by 350-fold upon the formation of thioethers. Probe **75a** was applicable to label proteins of extremely

low concentrations in gels.[87]

1.6.3 Other Functional BODIPY derivatives

The amine- and thio- reactive BODIPYs have been widely used to label purified biomolecules. These reagents have also found utility in non-selective *in situ* labeling of total cellular thiol or amine content.[12] There exists a need for bio-orthogonal *in situ* labeling that can be carried out under mild conditions with high degrees of selectivity.

The copper-catalyzed azide-alkyne cyloaddition, or the "Click" chemistry, is one of the most widely used bioorthogonal labeling reactions.[12] Wang and co-workers recently reported[88] the synthesis of a new type of BODIPY compound **76** that carries an azido group at the 3-position of the BODIPY core (Figure 1-21). The azido group quenches the fluorescence of the dye, however, the fluorescence is switched on upon reaction with alkynes to afford the corresponding triazole derivatives. In another report, monofunctional BODIPY dyes containing an azide **77a** and alkyne **77b** were prepared as pH sensors.[89] These analogues could potentially be used as bio-orthogonal probes.



76 **77a** **77b**

Figure 1-21 Structures of azido, alkynyl BODIPY derivatives

1.7 General perspectives and objectives

1.7.1 Stability of BODIPY in acidic and basic condition

Many BODIPY fluorophores possess high molar extinction coefficients and are highly fluorescent.[10, 11, 90] In addition, many BODIPY fluorophores have been reported to be relatively insensitive to changes in pH and polarity, and are more photochemically stable than many other commercially available fluorophores.[10, 11]

Although some reactivities of BODIPY have been documented in the literature,[10, 11] detailed studies on the stability of BODIPY have been rare.[91] One recent publication relates to the replacement of the fluorine atoms in BODIPY by hydroxyl in the presence of a strong Lewis acid such as aluminium chloride.[29] In another report, it was shown that BODIPY analogues could be converted to their corresponding dipyrrins when treated with potassium butoxide under microwave conditions.[92] It is noted that BODIPY has been given credit in numerous articles to be chemically stable, however, according to the observations made in our laboratory, BODIPY fluorophore partially degraded when it was incorporated into oligonucleotides by the phosphoramidite chemistry-based solid-phase synthesis, particularly during acid and base treatments.[93] Thus, the relative stability of various BODIPY analogues in acidic and basic conditions were compared in order to facilitate the selection of suitable BODIPY analogues for nucleic acid labeling *via* the phosphoramidite chemistry based solid phase synthesis approach.

1.7.2 Synthesis of the functional BODIPY derivatives

As mentioned above, various functional fluorophores can be introduced to different molecules. While BODIPY based fluorophores recently show an ever-growing success in

applications in fluorescent switches, and fluorophores in sensors and labels, it is worthwhile to expand the availability of activated BODIPY fluorophores for labeling target molecules. Herein we aim at the synthesis of azido- and isothiocyanate BODIPY derivatives. The latter will be transformed from the corresponding azido BODIPY.

Chapter 2 Synthesis of peptide nucleic acid

2.1 Peptide nucleic acid (PNA) – an artificial nucleic acid

DNA and RNA are carriers of genetic information. For the past 2-3 decades, there has been an increasing interest in the discovery and development of nucleic acid-based approaches for diagnosis and therapy.[94] In approaches such as antisense and RNA interference, some common features are required for the successful application of these nucleic acid-based therapies. Among these requirements, the therapeutic nucleic acids must bind to their target sequence with a desired affinity in a highly selective fashion. These molecules must also possess suitable stabilities, both chemically and enzymatically, so that they can reach their target sites before significant degradation takes place.

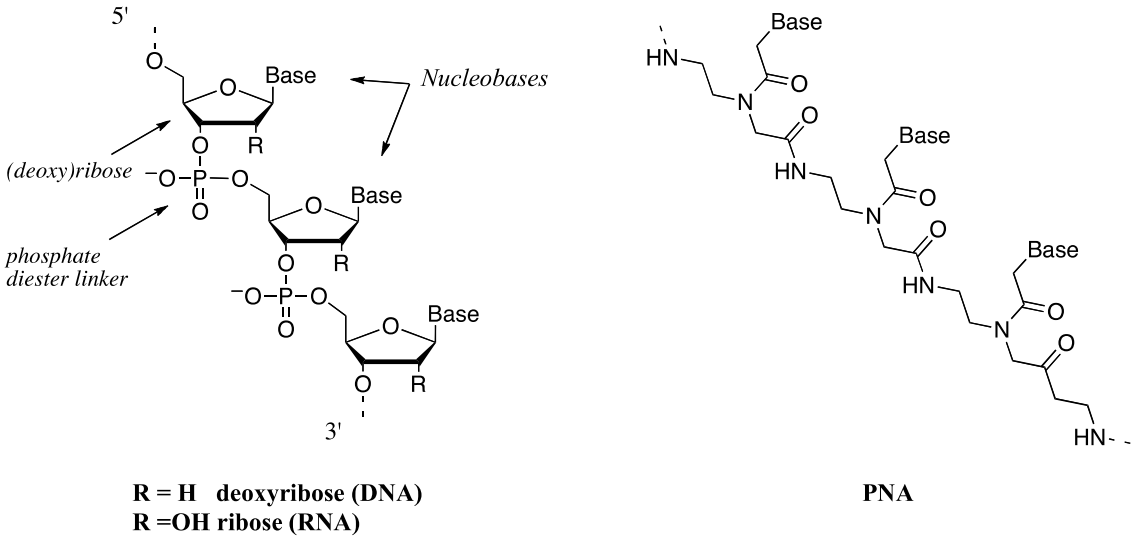


Figure 2-1 Native nucleic acids–DNA, RNA and PNA with *N*-(2-amino-ethyl)-glycine backbone

Peptide nucleic acid (PNA, Figure 2-1) meets all these requirements. PNA is designed as nuclease resistant DNA-binding ligands that can form stable and highly sequence-specific

complexes with DNA.[95] As a DNA mimic, PNA is structurally similar to DNA. Natural oligonucleotides, both DNA and RNA, have backbones with sugar-phosphate units while PNAs are built upon a pseudopeptide chain constituted by *N*-(2-aminoethyl)glycine monomers linked with the four nucleobases via methylene carbonyl bonds.

PNAs were first independently synthesized during the 1980s in Prof. Ole Buchardt's laboratories together with biochemist Peter Nielsen.[96] PNA is regarded as a fully synthetic DNA/RNA-binding ligand with a neutral achiral peptide-like backbone.

The pseudopeptide backbone confers PNAs with superior chemical and enzymatic stability. PNAs are resistant to enzymatic cleavage and thus not expected to degrade inside living systems.[97] On the other hand, PNA is capable of recognizing complementary DNA and RNA sequences to form stable duplex structures through Watson-Crick base pairing.[98] The hydrogen bonding and the hybrid complexes exhibit extraordinary thermal stability and unique ionic strength effects.

PNAs have been widely applied in molecular biology and biotechnology as useful tools in genetic diagnostics and specific regulations of gene expression.[99] They also have been extensively investigated as potential antiviral and anticancer drugs as well as antigene and antisense therapeutic agents.[97,99, 100]

2.2 PNA chemistry

PNA can be prepared in ways analogous to peptides, which involves coupling the carboxyl to the amino groups. Similarly, both solid and solution phase synthetic approaches can be utilized for PNA synthesis. In both approaches suitable protecting group strategies are crucial towards the synthesis of PNA monomers and subsequent oligomerization.

2.2.1 Amino-protecting groups

The α-amino group requires temporary protection in PNA chemistry. Some commonly used amino protecting groups are described below.

2.2.1.1 *tert*-Butoxycarbonyl (Boc)

The Boc protecting group can be readily removed upon treatment with strong acids such as trifluoroacetic acid, either neat or in dichloromethane, or with hydrogen chloride in methanol.

2.2.1.2 Fluorenylmethyloxycarbonyl (Fmoc)

The 9-fluorenylmethyloxycarbonyl (Fmoc) group is an alternative to the Boc group. The Fmoc protecting group can be deprotected by treatment with bases such as piperidine. The elimination product fulvene from the deprotection can often be trapped by a nucleophile such as thiol.

2.2.1.3 Benzyloxy-carbonyl (*Z*) group

Z Group is a widely used protecting group for amines because of its high stability in base and mild acid treatments and the versatile removal conditions. The Z protecting group can be removed by treatment of catalytic hydrogenolysis.[101]

2.2.2 Carboxyl protecting group

Protecting groups for carboxyls must be compatible with those for amino groups and any other protecting groups on nucleobase residues. Some commonly used carboxyl protecting groups are summarized below.

2.2.2.1 *tert*-Butyl (^{t}Bu) ester

tert-Butyl (^{t}Bu) group can be used as carboxylic acid protecting group in both solution and solid-phase synthesis of PNA. *tert*-Butyl esters are deprotected under the same conditions as Boc, *i.e*. by treatment with trifluoroacetic acid.

2.2.2.2 Benzyl (Bn) ester

Benzyl is one of the commonly used carboxylic acid protecting groups. The benzyl group can be cleaved by hydrogenation.

2.2.2.3 Allyl (All) group

The allyl (all) group can be used as an alternative for carboxylic acid protection. It can be readily removed by treatment with a catalytic amount of Pd(0), such as tetrakis(triphenylphosphine)palladium(0), in the presence of an appropriate nucleophile. The latter is used to trap the allyl palladium complex.

2.2.2.4 Methyl (Me) and ethyl (Et) ester

These simple esters can be cleaved by saponification, usually by treatment with lithium hydroxide or sodium hydroxide in methanol.

2.2.3 Coupling methods

The carboxy group on the PNA backbone is usually activated in the coupling step. The carboxyls can be either activated *in situ*, or prepared as the corresponding activated esters such as pentafluorophenyl esters.

2.2.3.1 Carbodiimides

In peptide bond formation, activated carboxylic acid derivatives are rarely stable. Thus the protected precursors are normally activated for coupling *in situ*. Carbodiimides such as

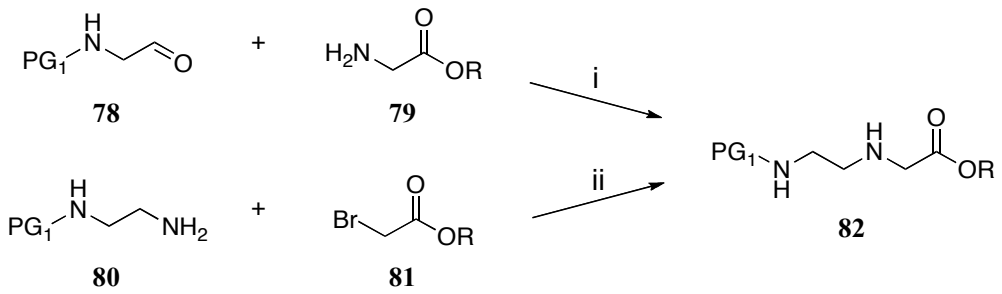
dicyclohexylcarbodiimide (DCC) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) are most commonly used as activating agents in these coupling reactions. EDC is used as an alternative to DCC because the by-product can be readily removed by extraction with water.

2.2.3.2 *N*-Hydroxy succinimide and triazole-based reagents

The *O*-isourea intermediates generated by treatment of carboxyls with carbodiimides are unstable and are often further treated with *N*-hydroxy succinimide and triazole analogues such as 1-hydroxy-benzotriazole (HOBt) and 1-hydroxy-7-aza-benzotriazole (HOAt). This treatment transforms the *O*-isourea intermediate into a less reactive, but more stable, reactive intermediate for peptide formation reactions. Benzotriazol-1-yl-oxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP) has also been used in this respect due to its stability and solubility, however the by-product hexamethylphosphoric acid triamide generated from BOP is carcinogenic.

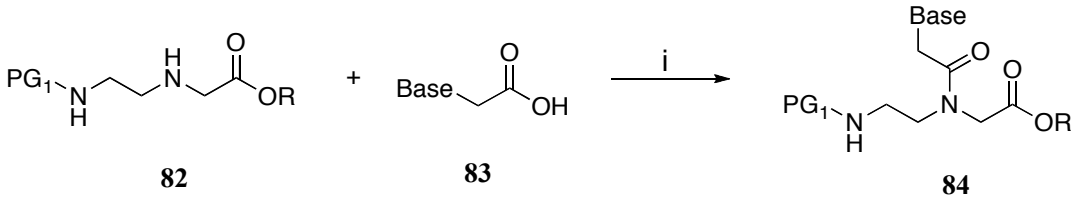
2.2.4 Synthesis of PNA monomer

As previously described, a PNA monomer consists of (2-aminoethyl)glycine backbone with a nucleobase attached. Two main routes have been used towards the synthesis of the backbone unit (Scheme 2-1). It is reported that the desired peptide backbone **82** can be obtained in high yields via either reductive amination of a glycine ester **79** with a suitably *N*-protected aminoacetaldehyde **78**,[102] or alkylation of mono-*N*-protected ethylenediamine **80** with a bromoacetic acid ester **81**.[103]



Scheme 2-1 Synthesis of protected (2-aminoethyl) glycine. *Reagents and conditions*: i) $NaBH_3CN$, MeOH; ii) K_2CO_3, $CHCl_3/CH_3CN$

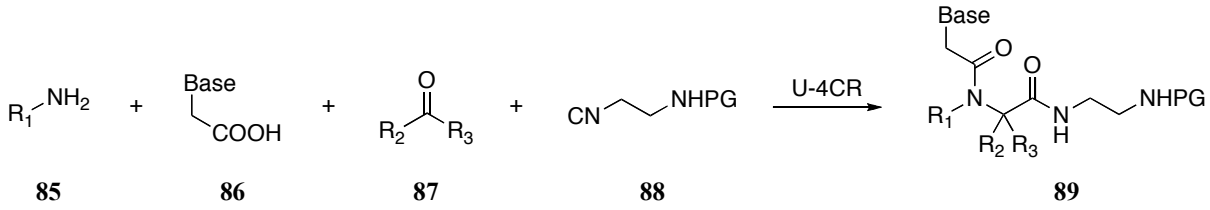
Suitably protected nucleobases **83** can then be attached to the PNA backbone via amide bond formation activated by coupling agents such as DCC,[104] EDC[105] and BOP[105, 106] (Scheme 2-2).



Scheme 2-2 Synthesis of PNA monomer. *Reagents and conditions*: i) DCC/EDC/BOP, DMF. PG: protected group.

Many PNA monomers are commercially available, however, they are very expensive. Commercial PNA monomers are also limited in variety. Some unusual or modified *N*-(2-aminoethyl)glycine derivatives have been developed. Hudson and co-workers[107] reported a convenient and scalable synthesis of *N*-(2-*N*-Boc-aminoethyl)glycinate based on the reductive amination of *N*-Boc-ethylenediamine with ethyl glyoxylate hydrate. The novel strategy took advantage of ethyl glyoxylate hydrate instead of traditional aldehyde to generate the desired backbone in virtually quantitative yield and high purity. Ugi reaction has also been shown for the synthesis of PNA monomers (Scheme 2-3).[108] Thus, PNA monomers **89** were prepared by treating nucleobase acetic acids **86** with primary amines **85**, oxo-components **87** and *N*-

mono protected ethylenediamine derived mono isocyanides **88**, in a four component fashion. Xu *et. al*. utilized this method to obtain not only PNA monomers but also PNA chain elongation products.[108d]



85 **86** **87** **88** **89**

Scheme 2-3 One-pot synthesis of PNA monomer via the four component Ugi reaction.

2.2.5 Synthesis of PNA oligomers

PNA oligomers can be prepared through either solid or solution phase synthesis. The solid phase synthesis has become the preferred method, especially for small-scale preparations, due to the simplicity in this approach compared with solution phase synthesis.

Solid phase synthesis of PNA is rather similar to that of peptides, which can be carried out either from the C to N or N to C termini. The synthesis cycle involves the following steps: 1) chain elongation (coupling), 2) washing, 3) removal of the transient protecting group and 4) washing.[109]

Functionalized polystyrene is among the most commonly used solid support. *N*-Protected amino acid (*e.g*. glycine) is attached to the solid support via the C-terminus to a cleavable linker. The choice of linker is highly relevant to the protecting group strategy used. For instance, the Wang resin, 4-(hydroxylmethyl)phenol, is applicable to the Fmoc/ benzhydryloxycarbonyl (Bhoc) chemistry. The final product can be cleaved from the resin by the treatment with acid such as TFA. While HMBA (4-(hydroxymethyl)benzoic acid linker) linker is relatively more stable under acidic condition so that it can be used for

monomethoxytrityl (MMT)/Acyl chemistry.

2.3 Comparison of PNA with DNA and RNA

Compared with DNA and RNA, PNA exhibits a better chemo-stability due to the polyamide-based backbone. The amide bond is stable under acidic and basic conditions. Thus PNA shows great stability over a wide pH range. PNA is resistant to degradation by enzymes such as nucleases, proteases or peptidases.

PNA binds complementary DNAs with affinity that is often higher than DNA duplexes, partially due to the neutral PNA backbone that reduces the electrostatic repulsion between phosphate negative charges. PNA/PNA duplexes are stable. Unlike DNA duplexes, PNA/PNA duplexes are not affected by the ionic strength of the media. PNA/DNA duplexes show greater specificity compared with DNA duplexes. As such, PNAs are very useful in discriminating single base mismatches.

On the other hand, the neutral backbone also leads to low water solubility of PNA. PNA has a tendency to aggregate, which is also attributed to the neutral amide backbone. The degree of aggregation is sequence dependent.[96] The low solubility of PNA can be mitigated to some extend by the addition of solubility enhancers.[110] PNA solubility also depends on purine/pyrimidine base ratio and the size of PNA oligomer.[111] Much like DNAs, PNAs have rather poor cellular permeability, which limits its efficiency in applications such as antigene or antisense therapies.[112]

2.4 Applications of PNA

Due to its unique properties, PNA has been explored for its potential in applications such as antisense and antigen therapeutics, molecular biology tools and diagnostics.

2.4.1 Antigene and antisense therapeutic agents

The potential in sequence-specific modulation of gene expression makes PNA a promising candidate as antisense and antigen therapeutic agent. In principle, several strategies were applied to design PNA based gene therapeutic drugs, including transcription arrest (antigen strategy),[113] translation arrest (antisense strategy),[114] and inhibition of replication.[115]

Transcription arrest can be achieved through the formation of a stable triplex, a strand-invasion or a strand displacement complex with DNA. Under transcription arrest, RNA polymerase is inhibited due to the hindrance from the PNA/$(DNA)_2$ triplex. One of the major obstacles for its *in vivo* application as antigen agents is that PNA strand invasion is dependent on salt conditions. Under moderate salt concentration, PNA strand invasion occurs at a very low rate.[100b]

In the antisense approach, binding of oligonucleotides to messenger RNA leads to translational arrest.[116] PNA shows tighter and more specific binding to RNA compared with DNA/RNA duplex. Thus PNA is regarded as an excellent candidate in antisense drug development. Nielsen's group showed that C-rich ($C_4T_4CT_4$) and T-rich PNAs (TC_6T_4CT) specifically arrest type 1 human immunodeficiency virus *in vitro*.[114b]

2.4.2 PNA probes in diagnosis and detection

PNA binds to nucleic acids with specificity higher than DNA, sensitivity and accuracy, and have thus been considered as an extraordinary candidate for various genetic diagnostic techniques. For instance, the PNA-based fluorescence *in situ* hybridization (FISH) technique has provided an attractive alternative for application in quantitative analysis of telomere length, chromosome painting and bacterial identification.[117] Additionally, PNA-mediated

PCR clamping technique is favored for identification of genetic diseases.[118] Furthermore, PNA has been exploited in applications such as molecular beacons.[119]

2.4.3 PNA as tools in molecular biology and functional genomics

PNA also has exhibited potentials for use as tools in molecular biology and functional genomics.

PNA in combination with methylases and restriction endonucleases can be used to cleave DNA in a site-specific manner. This method could mitigate the problem of DNA cleavages at more than one site when restriction enzymes are used alone. In this approach, the PNA target site overlaps with the methylation/restriction enzyme site and prevent methylation of DNA by methylases, where unbound DNA sequences are methylated. Upon PNA-DNA dissociation, cleavages by restriction enzymes at a single site can be effected.[96, 120]

PNA is also very useful in the purification of nucleic acids. For instance, it was shown that target nucleic acid can be purified by PNA linked to His-tag that is immobilized on nickel affinity columns.[120]

In conclusion, due to its extraordinary properties, PNA has found a wide range of applications and is considered an excellent candidate in therapeutic applications.

2.5 General perspectives and objectives

PNA is generally prepared by the coupling reaction between amino and carboxyl groups. This approach is versatile, however, can give rise to products in low yields, especially for guanylate PNA analogues.

The Staudinger ligation was shown to be a useful method to couple a peptide with a C-terminal phosphinothioester to another peptide with an N-terminal alpha-azido group in a

number of publications.[121, 122] Work described in this thesis attempted to explore the utility of this reaction in the preparation of short PNA sequences, and in the long term ligation of oligomers.

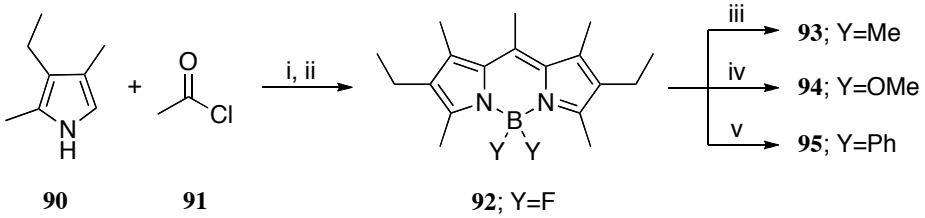
Chapter 3 Results and Discussion

Synthesis of BODIPY analogues

3.1 Overview of stability test of BODIPY in acidic and basic condition

Fluorescently labeled oligonucleotides have found a wide range of applications.[123] Towards this end, our group successfully prepared BODIPY phosphoramidites and then incorporated them into oligonucleotides by the phosphoramidite-chemistry based solid-phase synthesis.[124] In this approach, degradation of BODIPY derivatives was observed during the detritylation and amminolysis steps. This led us to evaluate the stability of BODIPY analogues under the conditions under which BODIPY is incorporated into oligonucleotides using the phosphoramidite chemistry.

4,4-Difluoro-4-bora-1,3,5,7-tetramethyl-2,6-diethyl-8-methyl-3a,4a-diaza-*s*-indacene **91** was chosen as a model for the stability experiments, as the subsitutents on the dipyrromethene ring are necessary to avoid potential reactions at these sites. This compound was readily prepared using a literature procedure by the treatment of 2,4-dimethyl-3-ethylpyrrole **90** with acetyl chloride **91**, followed by complexation with boron trifluoride diethyl etherate in the presence of triethylamine.[125] In addition, three other analogues, *i.e*. 4,4-dimethyl **93,**[126] 4,4-dimethoxy **94**[127] and 4,4-diphenyl **95**[128] BODIPY were prepared according to methods documented in the literature (Scheme 3-1).



90 **91** **92**; Y=F

Scheme 3-1. *Reagents and conditions*: i). CH_2Cl_2, reflux, 3 h; ii). CH_2Cl_2, NEt_3, $BF_3{\cdot}Et_2O$, reflux; iii). MeMgBr, CH_2Cl_2, 0 °C; iv). NaOMe, MeOH, CH_2Cl_2, reflux; v). PhMgBr, CH_2Cl_2, 0 °C.

3.1.1 Stability tests of BODIPY analogues in the presence of acid and base

3.1.1.1 Stability under acidic conditions

The stability of BODIPY fluorophores **92-95** in acidic solutions at room temperature was followed by recording ^{11}B NMR spectra at appropriate intervals. A sealed capillary insert of a solution of sodium tetraphenylborate in D_2O was used as an internal standard. In the case of an unstable substrate, the disappearance of substrate was monitored. In order to eliminate the broad ^{11}B resonance signal from the NMR tube, which overlaps with both substrate and internal standard signals, a linear prediction method [129] was used to process the ^{11}B NMR data. As shown in Figure 3-1, this linear prediction method allows for the suppression of background signals from the NMR tube.

The following processing parameters were used for the linear backward prediction algorithm:

Lb = 10 Hz

ME_mod = LPbr

NCOER = 32

LPBIN = 1024

Tdoff = 32

FCOR = 1

PKNL = TRUE

FT_mod = FSC

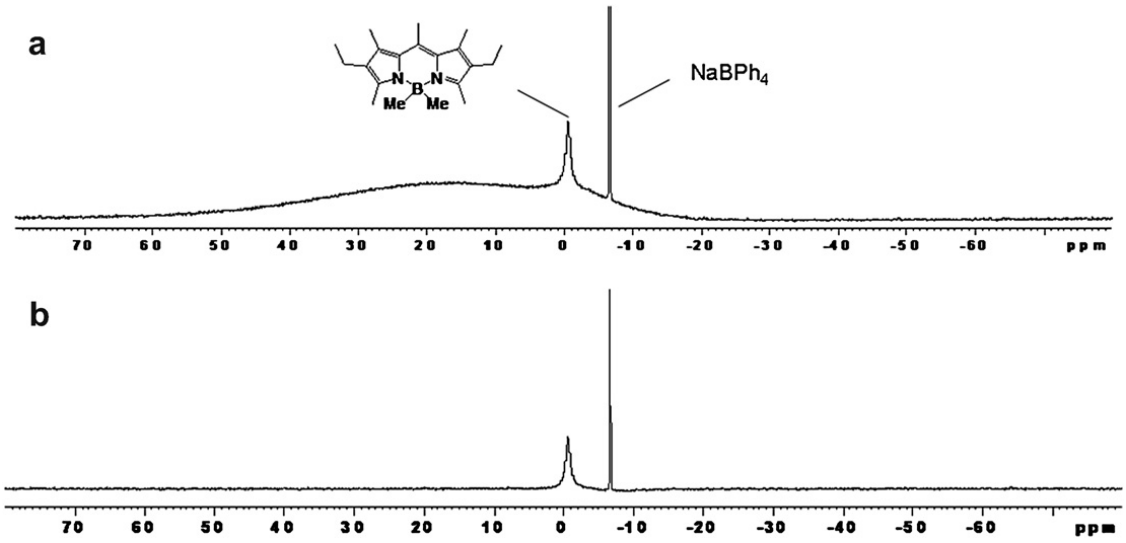


Figure 3-1 ^{11}B NMR spectra of **87** recorded in the presence of an internal stand ($NaBPh_4$). a) Before applying the linear backward prediction algorithm, and b) after applying the linear backward prediction algorithm.

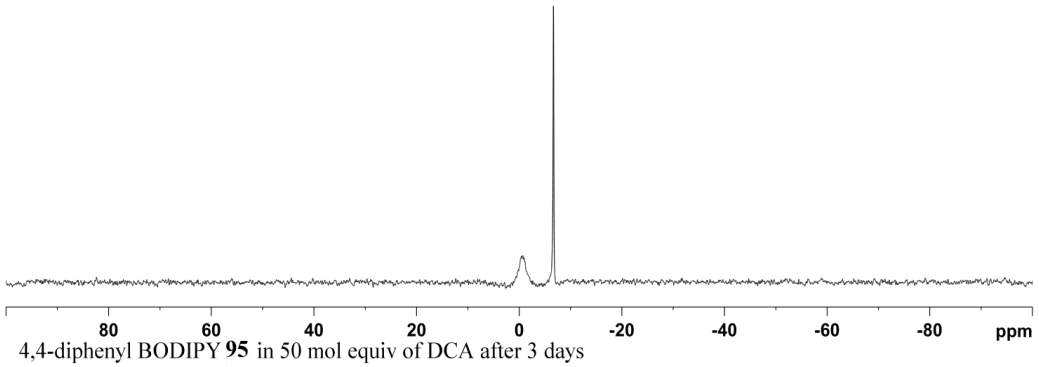
3.1.1.2 Stability under basic conditions

Stability tests of BODIPY analogues in basic conditions were carried out in the same way as described above for acid stability experiments, except that bases were used instead of acids.

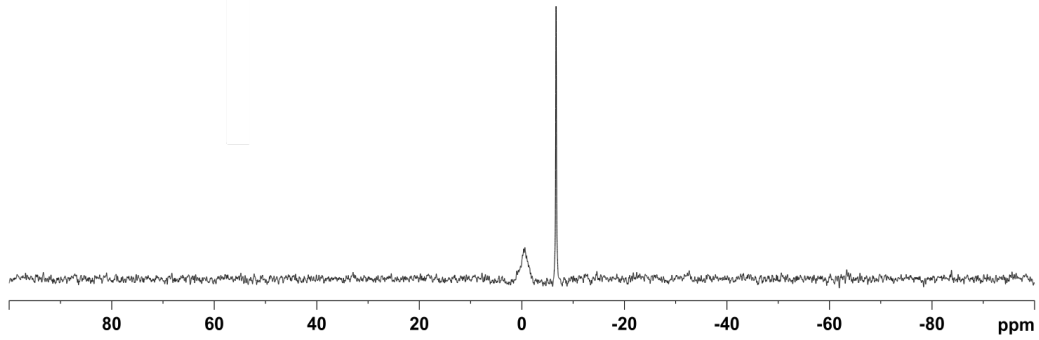
3.1.2 Stability results

It was found that 4,4-diphenyl BODIPY **95** was stable in a solution of dichloroacetic acid (50 mol. equiv.) in dichloromethane, indicated by the fact that no new signals appeared after 3 d (Figure 3-2a). 4,4-Difluoro BODIPY **92** is less stable under acidic conditions. When BODIPY **92** was treated with dichloroacetic acid (50 mol. equiv.) in dichloromethane, 8% decomposition was observed after 24 h. Treatment of 4,4-dimethyl BODIPY **93** under the same conditions led to complete decomposition in 6 hs to give a single boron-containing product, which was not identified (Figure 3-2b).

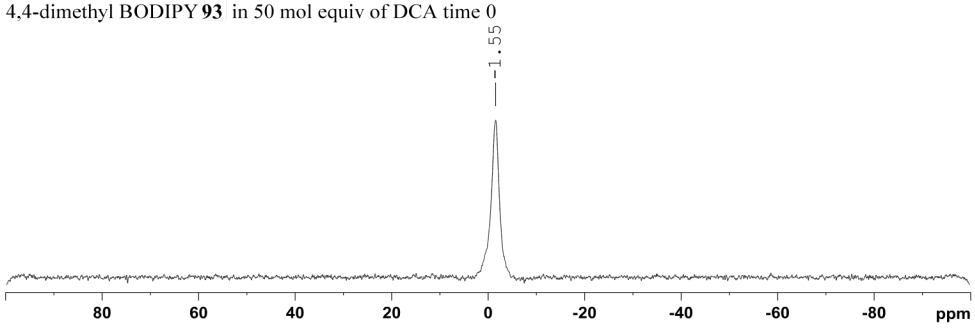
(a) 4,4-diphenyl BODIPY **95** in 50 mol equiv of DCA time 0



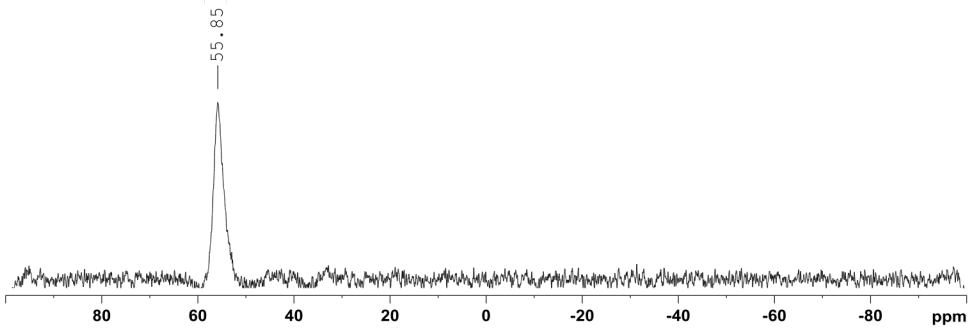
4,4-diphenyl BODIPY **95** in 50 mol equiv of DCA after 3 days



(b) 4,4-dimethyl BODIPY **93** in 50 mol equiv of DCA time 0



4,4-dimethyl BODIPY **93** in 50 mol equiv of DCA after 6 h



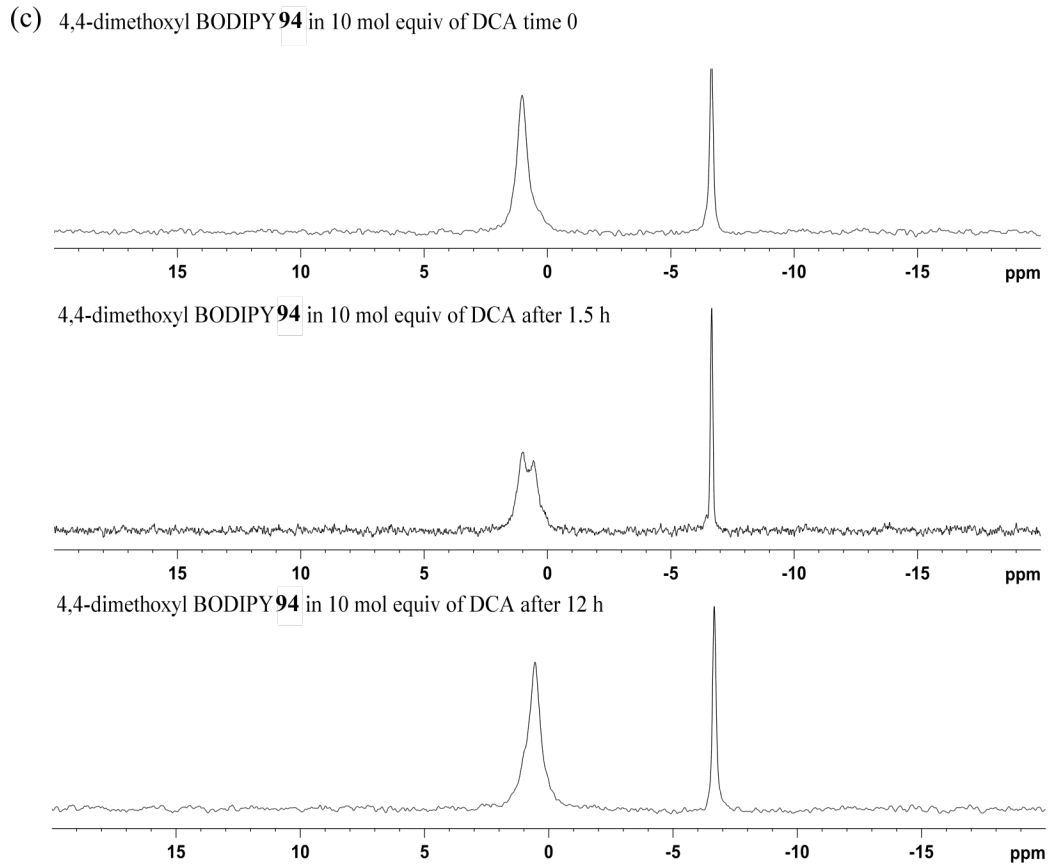
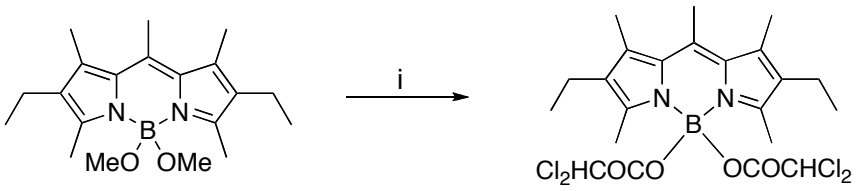


Figure 3-2 (a) **95** in 50 molar equivalents dichloroacetic acid-dichloromethane for 0 time and 3 days, respectively; (b) **93** in 50 molar equivalents dichloroacetic acid-dichloromethane for 0 time and 6 hours, respectively; (c) **94** in 50 molar equivalents dichloroacetic acid-dichloromethane for 0 time, 1.5 and 12 hours, respectively.

Interestingly, treatment of 4,4-dimethoxy BODIPY **94** with 10 mol. equiv. of dichloroacetic acid in dichloromethane led to full conversion of **94** to a single product in 12 h (Figure 3-2c). When this reaction was carried out on a preparative scale (Scheme 3-2), the product was identified as the corresponding anhydride **96**. The identity of this product **96** was confirmed by X-ray crystallography (Figure 3-3)



94 **96**

Scheme 3-2 *Reagents and conditions*: i). CH_2Cl_2, $Cl_2CHCOOH$, r.t.

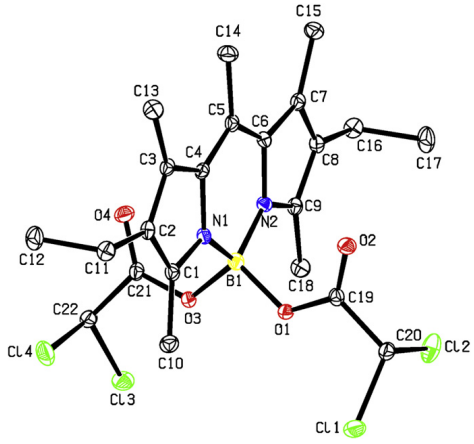


Figure 3-3 X-Ray crystal structure of 4,4-bis(dichloroacetoxy) BODIPY.

When a stronger acid, *i.e.* trichloroacetic acid (50 mol. equiv.), was used, both 4,4-difluoro **92** and 4,4-diphenyl **95** BODIPY became unstable. Thus, 50% of 4,4-difluoro BODIPY **92** undergoes decomposition after incubation in dichloromethane in the presence of 50 mol. equiv. of trichloroacetic acid for 3 h. Under the same conditions, 50% of 4,4-diphenyl BODIPY **95** undergoes decomposition in 4 h.

Stability of the BODIPY analogues under basic conditions was assessed as follows. The substrate was dissolved in tetrahydrofuran followed by addition of aqueous ammonium hydroxide and methanol (Methanol was not added in the case of 4,4-dimethoxy BODIPY **94**). THF and methanol were added in order to solubilize the substrates. Solutions of BODIPY analogues were then heated at 55^{o}C in sealed vials. At appropriate time intervals, samples were removed and ^{11}B NMR spectra were recorded.

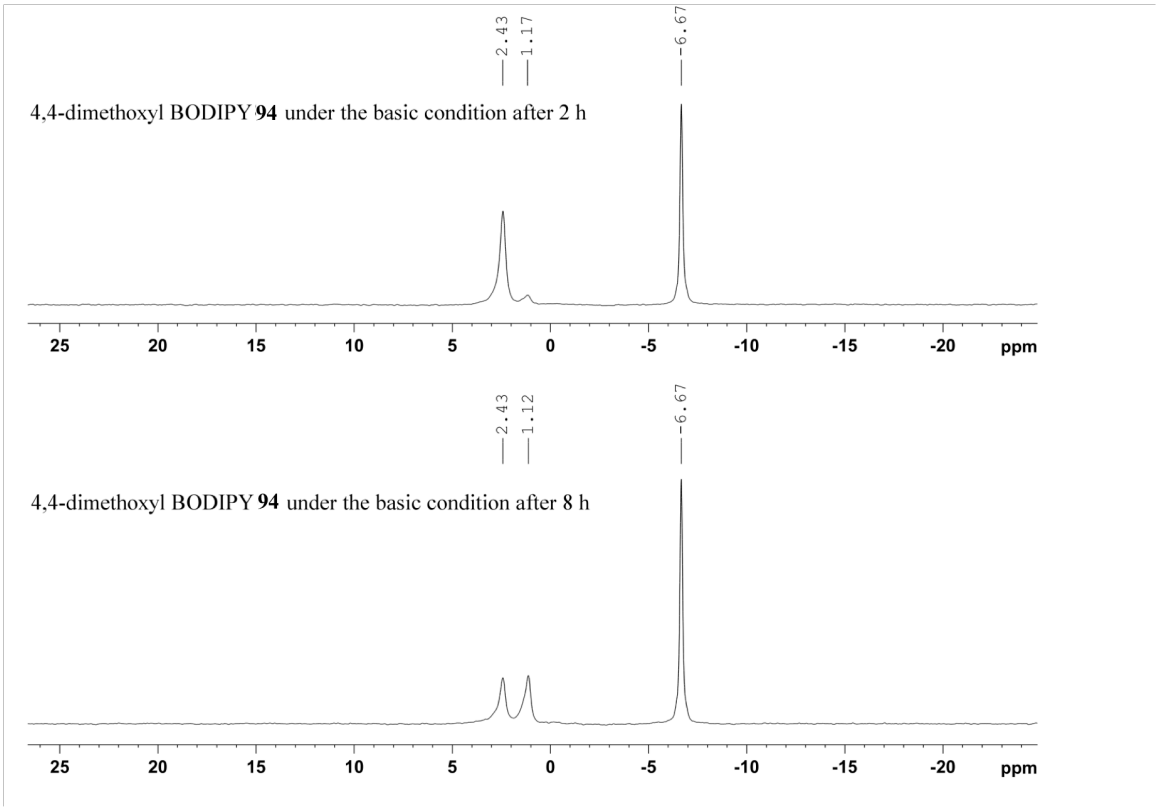


Figure 3-4 BODIPY **94** in dichloroacetic acid (50 mol. equiv.)–dichloromethane for 2 and 8 hs, respectively

The results showed that 4,4-dimethoxy BODIPY **94** is the least stable under this condition. Within about 8 h, half of the starting materials degraded, forming a product with a ^{11}B shift of 1.2 ppm (Figure 3-4). Under this condition 4,4-dimethyl **93** and 4,4-difluoro BODIPYs **92** are stable for up to 8 h, 4,4-diphenyl BODIPY **95** is stable for 7 days.

3.1.3 Conclusions

From the results obtained in this study, 4,4-diphenyl substituted BODIPY **95** appears to be the most stable under both acidic and basic conditions. It is also noted that substitution of the fluorine by phenyl does not compromise the fluorescent intensity of the fluorophore (Φ = 0.91 in dichloromethane for **95** and Φ = 0.83 for **92**).[11] The phenyl analogue **95** shows a larger Stoke's shift (35 nm) than the fluoro-BODIPY **92** (21 nm).[11] It is possible that the 4,4-

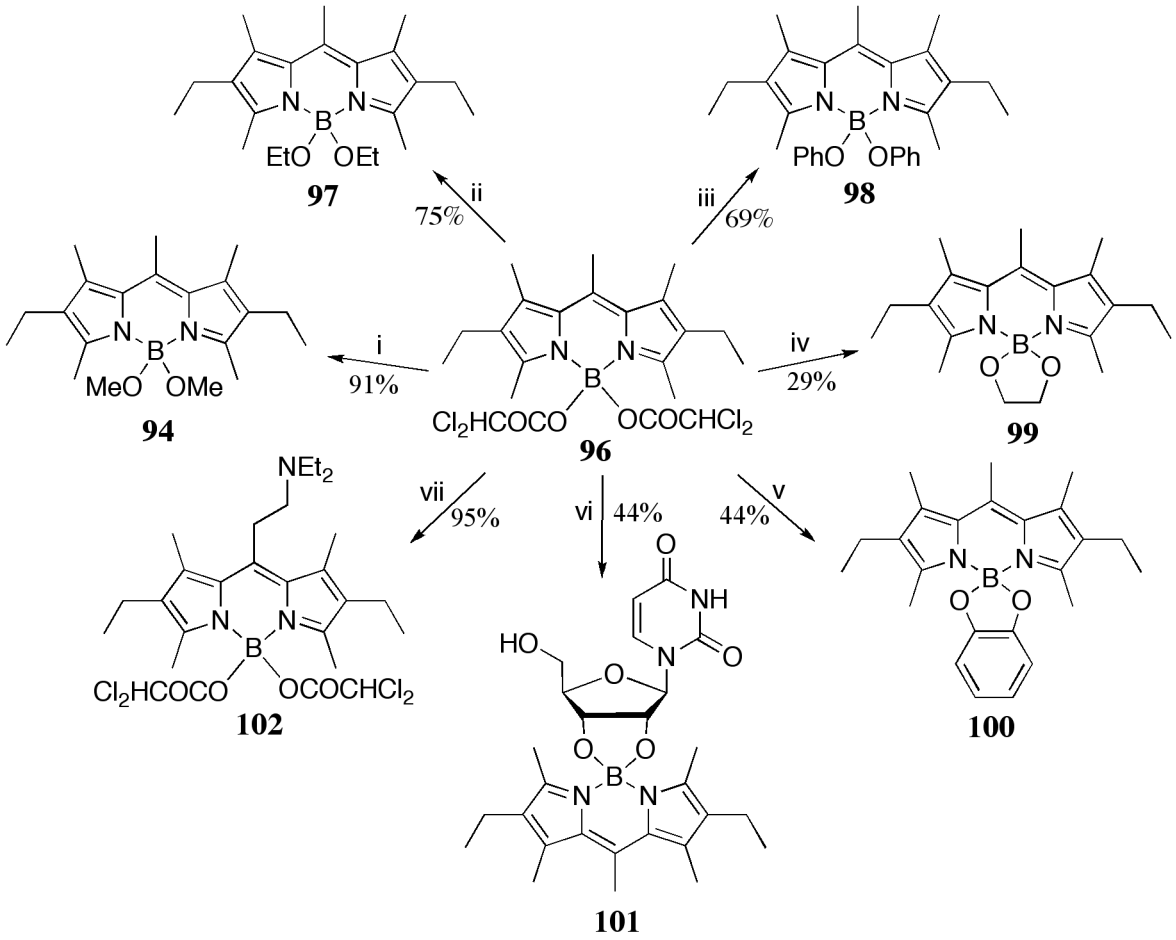
diphenyl substituted BODIPY analogues could be more suitable for oligonucleotide labeling via the phosphoramidite chemistry-based solid phase synthesis. Work in this direction will be explored in our laboratory in the future.

3.2 Some reactions of a 4,4-bis(dichloroacetoxy)BODIPY mixed anhydride

During the course of the evaluation of the stability of some BODIPY analogues, it was found that when 2,6-diethyl-4,4-dimethoxy-1,3,5,7,8-pentamethyl-4-bora-3a,4a-diaza-*s*-indacene **94** was treated with dichloroacetic acid, the corresponding mixed anhydride 4,4-bis(dichloroacetoxy)-2,6-diethyl-1,3,5,7,8-pentamethyl-4-bora-3a,4a-diaza-*s*-indacene **96** was formed (Scheme 3-2) and isolated in good yield. The synthetic utility of this new BODIPY species was explored.

When the mixed anhydride 4,4-bis(dichloroacetoxy)-2,6-diethyl-1,3,5,7,8-pentamethyl-4-bora-3a,4a-diaza-*s*-indacene **96** was treated with simple alcohols alone, such as methanol and ethanol, virtually no reaction was observed. However, in the presence of a strong base, such as 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), rapid formation of 2,6-diethyl-4,4-dimethoxy (or 4,4-diethoxy)-1,3,5,7,8-pentamethyl-4-bora-3a,4a-diaza-*s*-indacene **94** (or **97**) was observed, and the resulting products can be isolated in good yields (91%) by column chromatography (Scheme 3-3). Similar results were observed when phenol was used as a substrate in the presence of DBU, resulting in the formation of 2,6-diethyl-4,4-diphenoxy-1,3,5,7,8-pentamethyl-4-bora-3a,4a-diaza-*s*-indacene **98** in 69% yield. If a 1,2-diol is used instead of a monofunctional alcohol, a cyclic borate is formed. Thus, treatment of 4,4-bis(dichloroacetoxy)-2,6-diethyl-1,3,5,7,8-pentamethyl-4-bora-3a,4a-diaza-*s*-indacene **96** with ethylene glycol and catechol in the presence of DBU gave corresponding cyclic borate esters **99** and **100** in 29 and 44% yield, respectively. Interestingly, when **96** was allowed to

react with uridine in the presence of DBU in *N*,*N*-dimethylformamide (DMF), uridine was labeled with BODIPY through the formation of a 2',3'-cyclic borate ester **101**.



Scheme 3-3 *Reagents and conditions*: i). CH_3OH, DBU, CH_2Cl_2; ii). EtOH, DBU, CH_2Cl_2; iii). PhOH, DBU, CH_2Cl_2; iv). $HOCH_2CH_2OH$, DBU, CH_2Cl_2; v). catechol, DBU, THF; vi). uridine, DBU, DMF; vii). $HNEt_2$, CH_2Cl_2.

Surprisingly, when 4,4-bis(dichloroacetoxy)-2,6-diethyl-1,3,5,7,8-pentamethyl-4-bora-3a,4a-diaza-*s*-indacene **96** was treated with diethylamine in dichloromethane, 4,4-bis(dichloroacetoxy)-2,6-diethyl-8-(2-(*N*,*N*-diethylamino)ethyl)-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene **102** was isolated in 41% yield. It was found, however, that this reaction was not observed when dichloromethane was replaced with tetrahydrofuran as

solvent. This observation clearly suggests the involvement of dichloromethane in the reaction. The nature and scope of this chemistry is discussed below.

In conclusion, 4,4-bis(dichloroacetoxy)-2,6-diethyl-1,3,5,7,8-pentamethyl-4-bora-3a,4a-diaza-*s*-indacene **96** as a reactive intermediate can be readily prepared and isolated by column chromatography and further used for the preparation of a series of BODIPY derivatives.

3.3 A Mannich-Type Reaction at the *meso*-Methyl Position of the BODIPY Fluorophore

As is described in the previous section, 4,4-bis(dichloroacetoxy)-2,6-diethyl-8-(2-(N,N-diethylamino)ethyl)-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene **102** was isolated as a major product when 4,4-bis(dichloroacetoxy)-2,6-diethyl-1,3,5,7,8-pentamethyl-4-bora-3a,4a-diaza-*s*-indacene **96** was treated with diethylamine in dichloromethane.

It was recognized in the literature that dihalomethanes and secondary amines (and tertiary amines) react readily. For instance, triethylamine and dichloromethane react to form *N*-chloromethylammonium.[130] Similar reactions were also documented elsewhere.[131] It was proposed in the literature[132] that treatment of dichloromethane with dimethylamine gave *N*,*N*,*N*′,*N*′-tetramethylmethylenediamine (Scheme 3-4). This hypothesis was later confirmed experimentally.[133] Formation of an iminium salt was subsequent suggested in the literature.[133a] Indeed when ^{1}H NMR of a solution of diethylamine in deuterated dichloromethane were recorded over 48 h, formation of a new species was observed (Figure 3-5).

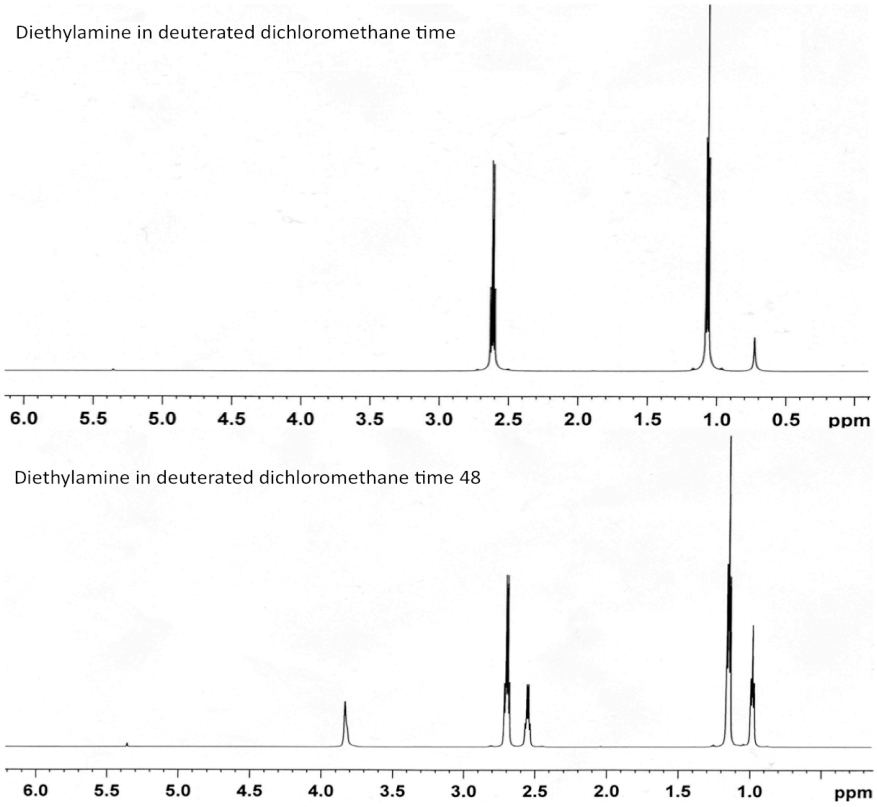
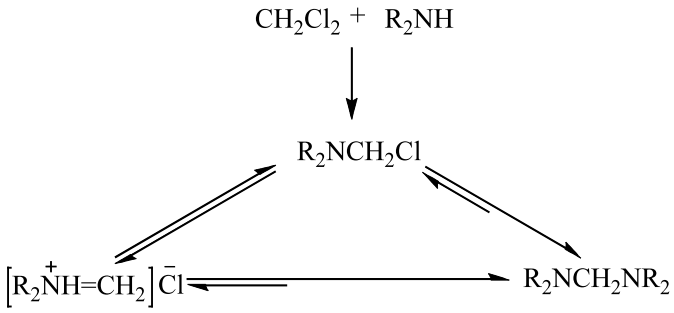


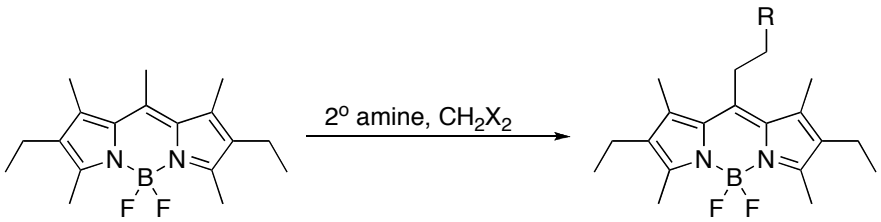
Figure 3-5 Diethylamine in deuterated dichloromethane at 0 and 48 hs, respectively.



Scheme 3-4 Proposed pathway for reactions between secondary amines and dichloromethane.[132]

When 2,6-diethyl-4,4-difluoro-1,3,5,7,8-pentamethyl-4-bora-3a,4a-diaza-*s*-indacene **92** was subjected to treatment with secondary amines in dihalomethanes **103** (Scheme 3-5), Mannich-type reactions were observed. Depending on the steric bulk and basicity of the secondary amine, either dichloromethane or dibromomethane can be used in these reactions

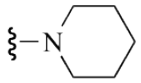
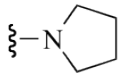
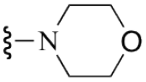
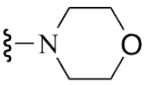
(Table 3-1). In all cases, the Mannich products **103** were readily isolated in moderate to good yields (Table 3-1).



92 **103**

Scheme 3-5 Mannich reactions of 4,4-difluoro BODIPY with various secondary amines.

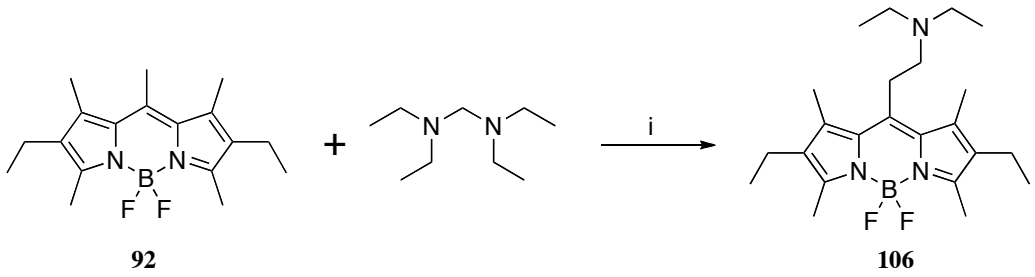
Table 3-1 Mannich reaction (Scheme 3-5) products

Entry	2	R	CH_2X_2	Temp (°C)	Time (d)	Yield (%)
1	**87**	NEt_2	CH_2Cl_2	40	3	62
2	**88**	NMe_2	CH_2Cl_2	40	4	61
3	**89**	$N(i\text{-}Pr)_2$	CH_2Cl_2	40	7	34
4	**89**	$N(i\text{-}Pr)_2$	CH_2Br_2	60	1	75
5	**90**		CH_2Cl_2	40	4	57
6	**91**		CH_2Cl_2	40	1	63
7	**92**		CH_2Cl_2	40	7	_a
8	**92**		CH_2Br_2	60	7	66

a. No product formation was observed by TLC after heating at 40 °C for 3 d.

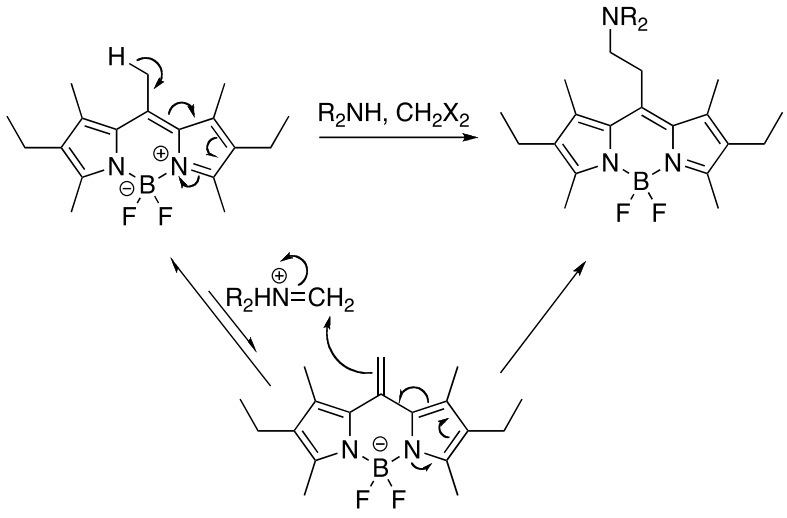
These observations are analogous to the Mannich reactions involving secondary amines and dihalomethanes that have been documented in the literature.[134] In connection with the pathway described in Scheme 3-5, a reaction between 2,6-diethyl-4,4-difluoro-1,3,5,7,8-pentamethyl-4-bora-3a,4a-diaza-*s*-indacene **92** and *N*,*N*,*N*′,*N*′-tetraethylmethylenediamine **110**

was carried out in THF (Scheme 3-6). As expected, the corresponding Mannich product **104** was isolated in good yield (93%).



92 **106**

Scheme 3-6 *Reagents and conditions*: i) THF, 40°C.



Scheme 3-7 A postulated mechanism for the Mannich-type reaction.

Based on these observations, a reaction mechanism is postulated as follows (Scheme 3-7). Upon deprotonation of BODIPY, the intermediate reacts with the highly electrophilic Mannich base, leading to carbon–carbon bond formation at the BODIPY *meso*-methyl position.

The UV/*vis* absorption and fluorescent emission spectra of **104** were recorded in acetonitrile and are shown in Figure 3-6. The other Mannich products **104–109** show very similar absorption and fluorescent profiles.

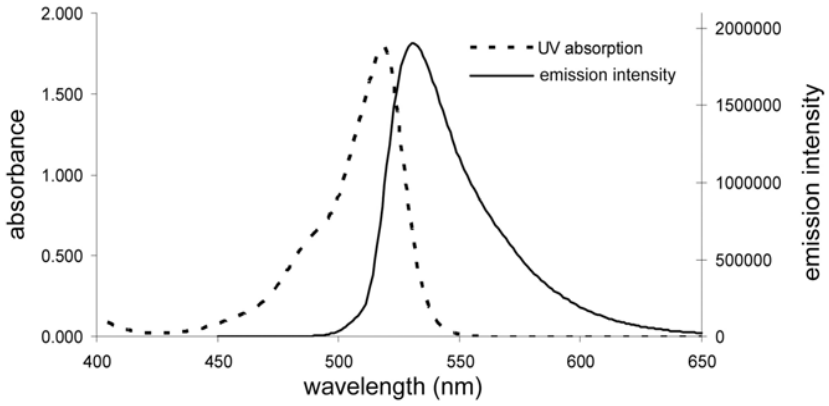


Figure 3-6 Absorption and fluorescence emission spectra (excited at 517 nm) of compound **104** in acetonitrile.

As can be seen from Figure 3-6, the Mannich product **104**, as well as **104–109**, shows rather typical BODIPY absorption and emission profiles, with small Stokes shifts. All products are highly fluorescent, with high fluorescent quantum yields (Table 3-2).

Table 3-2 UV/*vis* absorption, fluorescent emission maxima and fluorescent quantum yields of compounds **104**-**109** in acetonitrile.

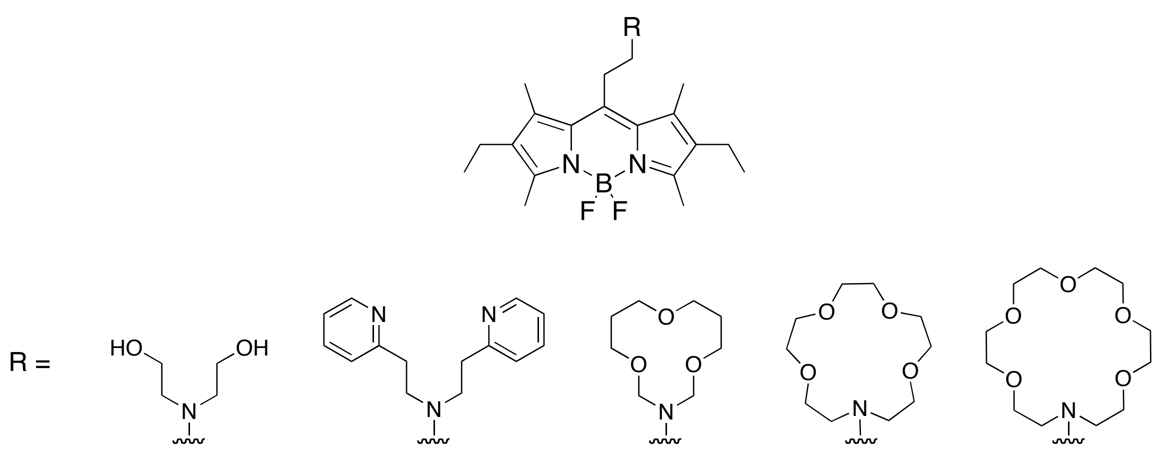
Entry	$\lambda_{max\ abs}$(nm)	$\lambda_{max\ em}$(nm)	ε ($\times 10^4$ cm^{-1} mol^{-1})	Φ [a]
104	517	530	7.30	1.0
105	518	530	6.59	0.80
106	514	530	6.57	0.90
107	517	530	7.16	0.86
108	518	531	7.23	0.85
109	519	530	7.01	0.75

[a]The fluorescent quantum yields were determined against fluorescein in 0.1 *M* NaOH, which has a quantum yield of 0.95±0.05 when excited at 496 nm.[135]

In conclusion, an unprecedented Mannich-type reaction between 2,6-diethyl-4,4-difluoro-1,3,5,7,8-pentamethyl-4-bora-3a,4a-diaza-*s*-indacene **92** and dihalomethane was found to be straightforward and gave corresponding *meso*-modified BODIPY Mannich products in moderate to good yields. This Mannich-type reaction can provide ready access to *meso*-substituted BODIPY analogues.

3.4 BODIPY-based fluorescent sensors for ions

It was shown that 8-methyl BODIPY **92** undergoes a Mannich-type reaction when treated with a secondary amine in dihalomethane, allowing for ready access to *meso*-substituted BODIPY. These results prompted us to investigate the utility of this Mannich chemistry in the preparation of BODIPY analogues that bear ion binders at the 8-position and to explore their applications in ion sensing.



111 **112** **113** **114** **115**

Figure 3-7 Structures of BODIPY based ion sensors.

Five BODIPY analogues **111-115** bearing substitution at the *meso*-position were synthesized in moderate yields from 2,6-diethyl-4,4-difluoro-1,3,5,7,8-pentamethyl-4-bora-3a,4a-diaza-*s*-indacene **92** and corresponding secondary amines under the Mannich reaction

conditions described in Scheme 3-5. It is noted that the conversion for the dihydroxyethylamine and pyridine derivatives were low and most of the unreacted starting material was recovered after the reaction was terminated.

3.4.1 Screening

Compounds **111-115** were screened at Professor Antai Wu's laboratory at the National Changhua University of Education in Taiwan against a panel of 12 cations (Ca^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+}, Hg^{2+}, K^{+}, Li^{+}, Mn^{2+}, Na^{+}, Ni^{2+}, Pb^{2+} and Zn^{2+}) and 7 anions (F^{-}, Cl^{-}, Br^{-}, I^{-}, HSO_4^{-}, NO_3^{-} and AcO^{-}). Experiments described in 3.4.1-3.4.2 were performed by De-Jhong Liao.

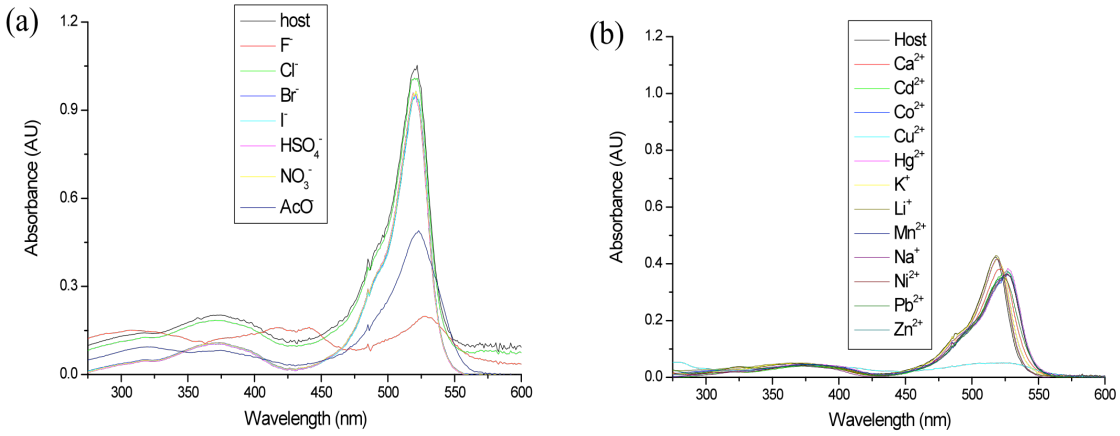


Figure 3-8 UV/*vis* spectra of **114** and **115** in the presence of various ions. a) UV/*vis* spectra of **114** recorded in DMSO (1.8×10^{-5} *M*) after addition of 10 equivalents of various anions; b) UV/*vis* spectra of **115** recorded in CH_3CN (8×10^{-6} *M*) after addition of 10 equivalents of various metal ions.

Among these five receptors, **114** and **115** were found to respond to selected ions and were furthered screened as sensor candidates. In the presence of F^{-}, a red shift with a hypochromic effect was observed in the UV/*vis* spectra of receptor **114** (Figure 3-8a) in DMSO, with a sharp decrease of absorbance at 527 nm and rising of a new peak at 438 nm. On the other

hand, from the UV/*vis* spectra, it was found that **115** (Figure 3-8b) showed a hypochromic effect in the presence of Cu^{2+} ion in acetonitrile.

The solution of receptor **114** showed a dramatic color change from pink to virtually colorless that could easily be seen with the naked eye (Figure 3-9a). When the solutions were irradiated with a UV lamp, the fluorescence difference of **114** can also be easily distinguished in the presence of various anions. (Figure 3-9b)

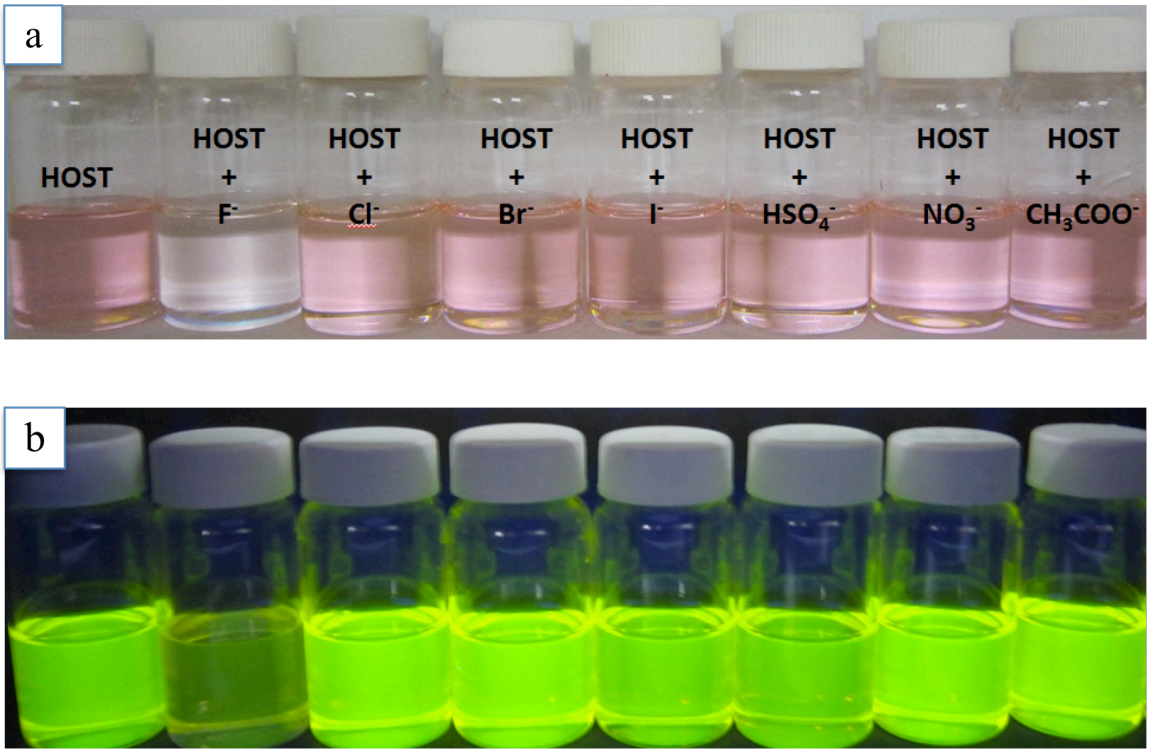


Figure 3-9 a) Naked eye detection of **114** upon addition of 10 equiv. of various anions in DMSO (5.6 μ*M*); b) fluorescent changes of sensor **114** in DMSO (5.6 μ*M*) after addition of 10 equiv. of anions (irradiated with UV lamp).

The chemosensing properties of receptors **114** and **115** were further investigated by fluorescent spectroscopy. Receptor **114** did not exhibit any selective recognition among a series of tested metal ions; however, it exhibited a highly selective recognition toward F^- in the presence of various anions in DMSO (Figure 3-10a), with a sharp decrease in emission at

550 nm. F^- ions also led to a blue shift (62 nm) in the fluorescent emission spectrum of **114**. On the other hand, the results indicated that the fluorescence of receptor **115** was strongly quenched in the presence of Cu^{2+} ion in CH_3CN (Figure 3-10b), where other metal ions did not significantly affect the fluorescence intensity of **115**. Thus the observed quenching efficiency by Cu^{2+}, which is calculated as $(I-I_0/I_0)\times100\%$ at 518 nm, was nearly 95% where the other metal ions only caused slight quenching in fluorescent emission intensity.

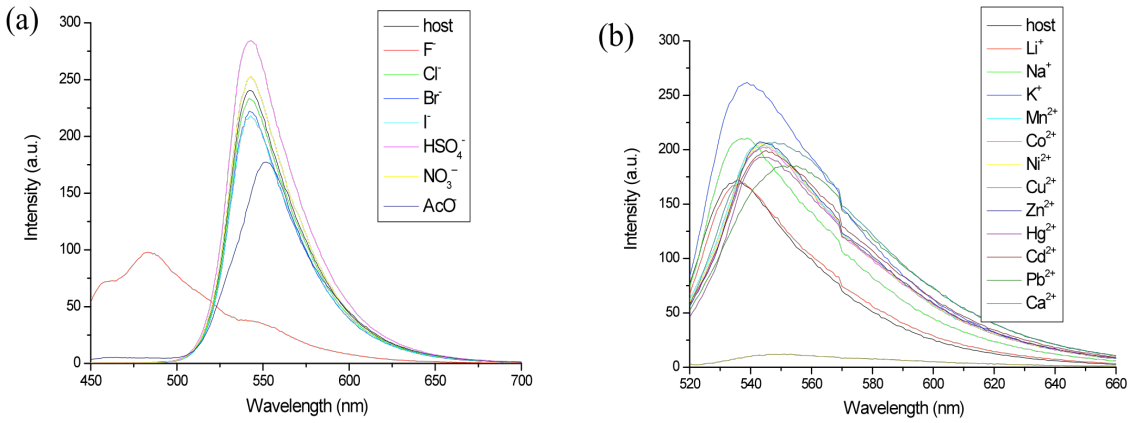


Figure 3-10 Fluorescence spectra of **114** and **115** upon addition of various ions. a) Fluorescence spectra of **114** (1.35×10^{-5} M) upon addition of various anions (10 equiv.) in DMSO. λ_{ex}= 375 nm; b) fluorescence spectra of **115** (8×10^{-6} M) upon addition of various cations (10 equiv.) in CH_3CN. λ_{ex}= 516 nm.

The fluorescence titrations of receptors **114** and **115** were performed in the presence of varying concentrations of F^- or Cu^{2+}, respectively. The fluorescence titration spectra of **114** in DMSO also showed decreases in fluorescent emission intensity in a F^- concentration-dependent manner (Figure 3-11a). Based on the fluorescence titration measurements, detection limit of receptor **114** for F^- was found to be 3.6×10^{-5} *M*. The association constant for **114***F^- complex was found to be 3.73×10^4 according to the Stern-Volmer plot (Figure 3-12a). The Job plot showed that receptor **114** formed a 1:1 complex with F^-. (Figure 3-12b)

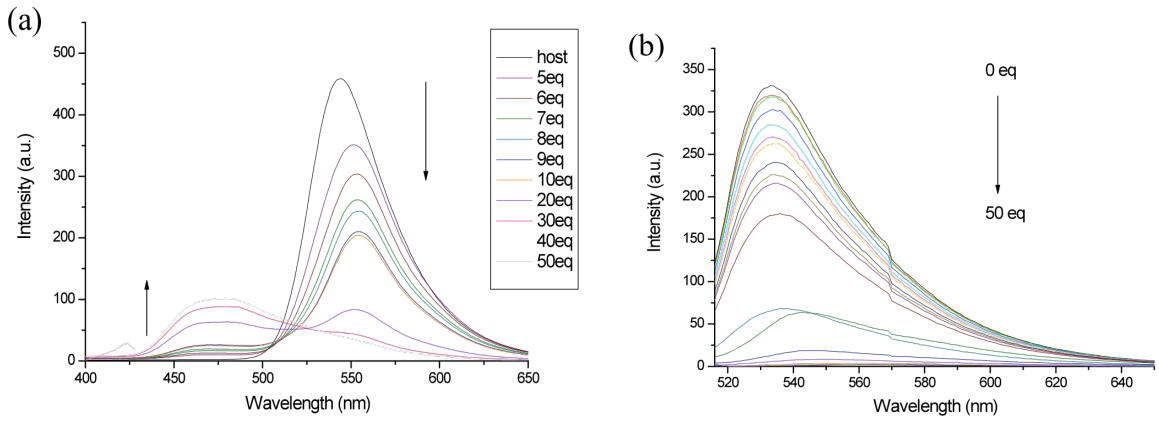


Figure 3-11 Fluorescence spectra of **114** and **115** in the present of varying concentrations of ions. a) Fluorescence spectra of **114** (7.2×10^{-6} *M*) in DMSO upon addition of increasing concentrations of F^-.; b) Fluorescence spectra of **115** (8×10^{-6} *M*) in CH_3CN upon addition of increasing concentrations of Cu^{2+}.

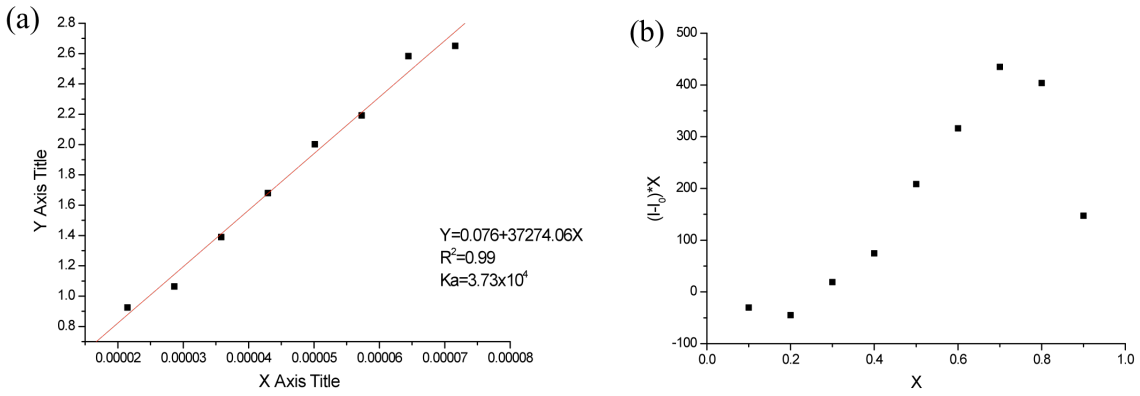


Figure 3-12 a) Stern-Volmer plot **of 114** with F^-; b) Job plot of a 1:1 complex of **114** with F^-.

Figure 3-11b showed the gradual reductions in fluorescence intensity for receptor **115** upon addition of Cu^{2+}. From the fluorescence titration profiles, the association constant for **115***Cu^{2+} was found to be 1.43×10^5, according to the Stern-Volmer plot (Figure 3-13a). Based on the fluorescence titration measurement, detection limit of receptor **115** for Cu^{2+} was found to be 2.4×10^{-6} *M*. In the Job's plot (Figure 3-13b), a maximum fluorescence change

was observed with a 0.5 molar fraction of receptor to Cu^{2+} ion for **115**, which indicated the formation of a 1:1 complex.

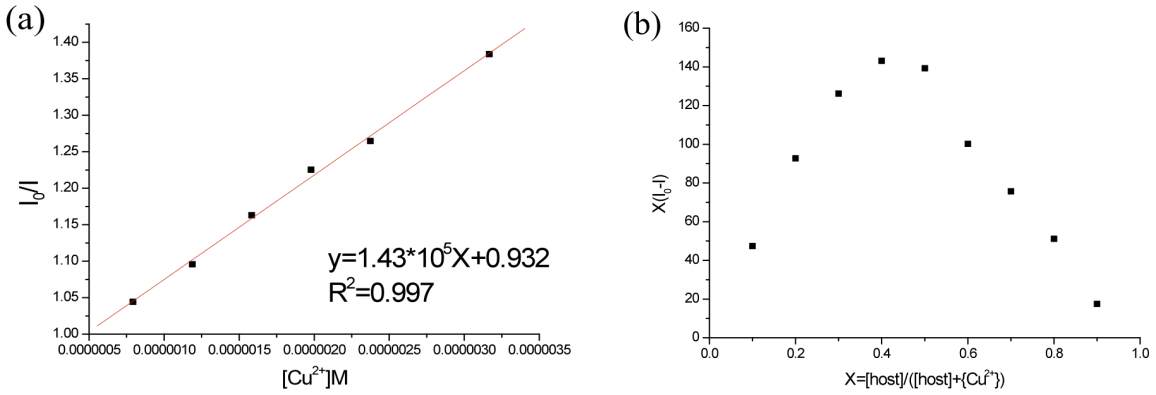


Figure 3-13 a) Stern-Volmer plot of **115** with $Cu(ClO_4)_2$; b) Job plot of a 1:1 complex of **115** (8×10^{-5} *M*) with Cu^{2+}.

3.4.2 Determination of selectivity by competition experiments

Selectivity of receptor **114** for F^- ion was further ascertained with competition experiments (Figure 3-14a). It was found that the fluorescence intensity of receptor **114** in the presence of 10 equivalents of F^- ion was unaffected by the addition of 10 equivalents of competing anions (Cl^-, Br^-, I^-, NO_3^-, HSO_4^-, AcO^-). This observation indicates that **114** is selective towards the recognition of F^- ion over other metal ions. For receptor **115,** it can be seen from the competition experiment results (Figure 3-14b) that the fluorescence intensity of receptor **115** in the presence of 10 equivalents of ion Cu^{2+} was also unaffected by the addition of 10 equivalents of competing metal ions (Li^+, Na^+, K^+, Ca^{2+}, Mg^{2+}, Co^{2+}, Ni^{2+}, Hg^{2+}, Pb^{2+}, Cd^{2+}, or Zn^{2+}).

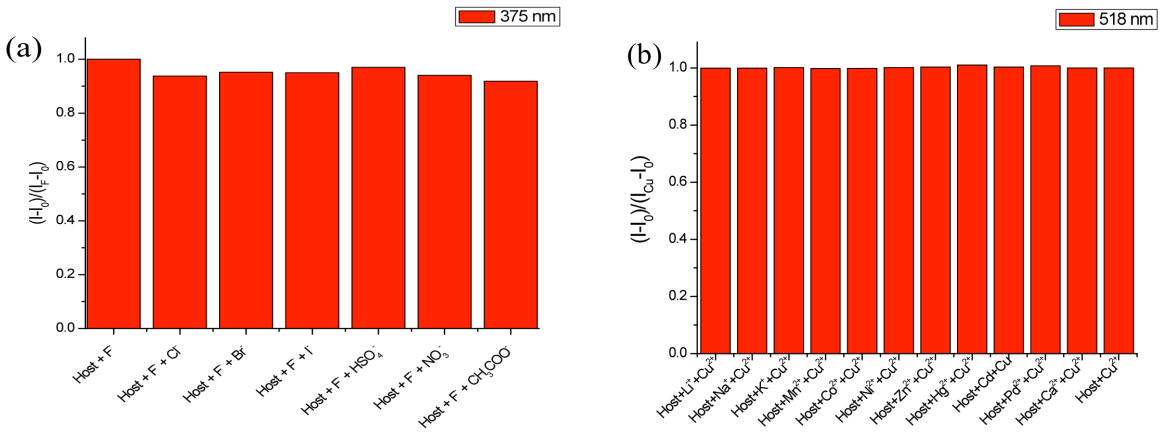


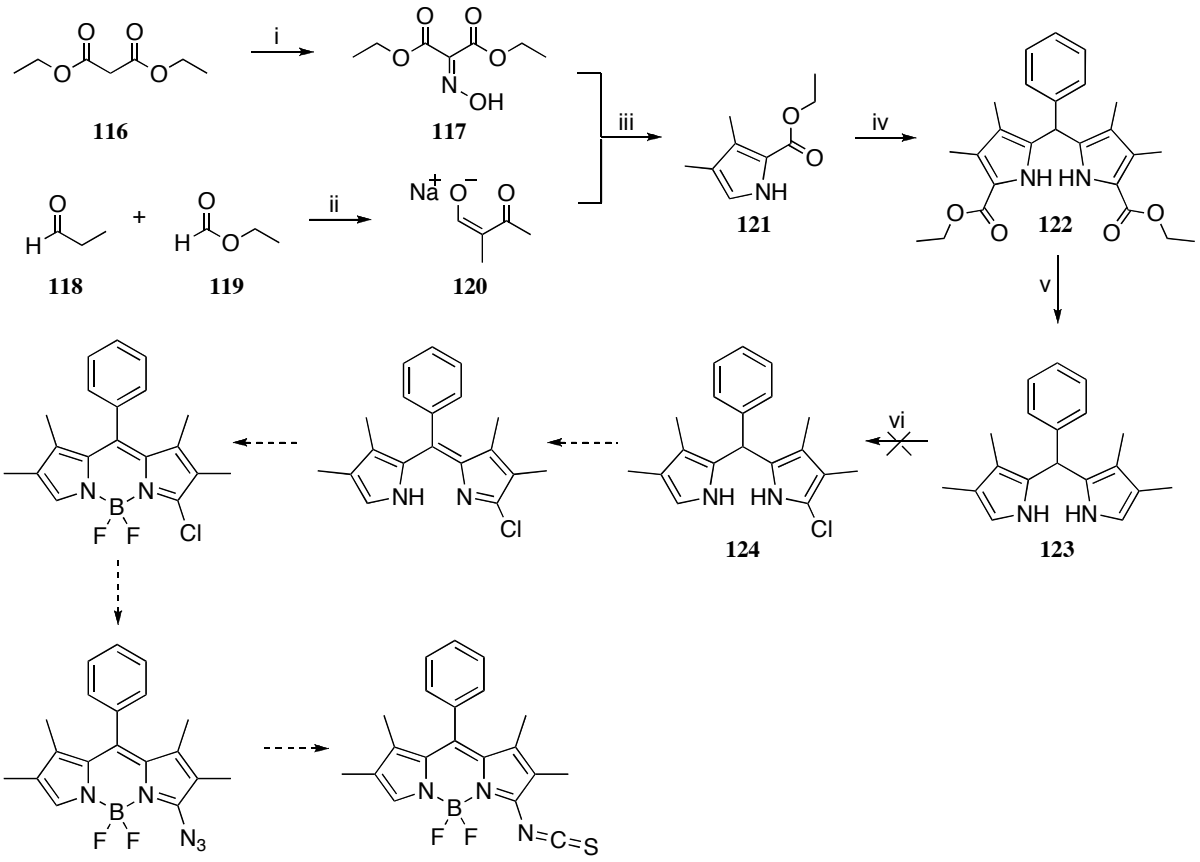
Figure 3-14 Competitive experiments of **114** and **115**. a) Competitive experiments in the **114** + F^- system with interfering anions. [**114**] = 7.2 μ*M*, [F^-] = 72 μ*M*, and [X^{n-}] = 72 μ*M* in DMSO. λ_{ex} = 375 nm; b) competitive experiments in the **115** + Cu^{2+} system with interfering metal ions. [**115**] = 80 μ*M*, [Cu^{2+}] = 800 μ*M*, and [X^+] = 800 μ*M* in CH_3CN. λ_{ex} = 518 nm

3.4.3 Conclusion

In summary, five BODIPY fluorophores modified at the *meso*-position were synthesized using the Mannich reaction that was developed in our laboratory. Among these, BODIPY bearing aza-15-crown-5 **114** and aza-18-crown-6 **115** modifications showed selective binding to F^- in DMSO and Cu^{2+} in acetonitrile, respectively, leading to ion concentration-dependent decreases in fluorescence emission intensity. However, the mechanism of the fluorescent quenching remains unknown.

3.5 Isothiocyanate and azide BODIPY

Syntheses of isothiocyanate and azide BODIPY were attempted in this thesis to expand the scope of BODIPY in labeling biomolecules.

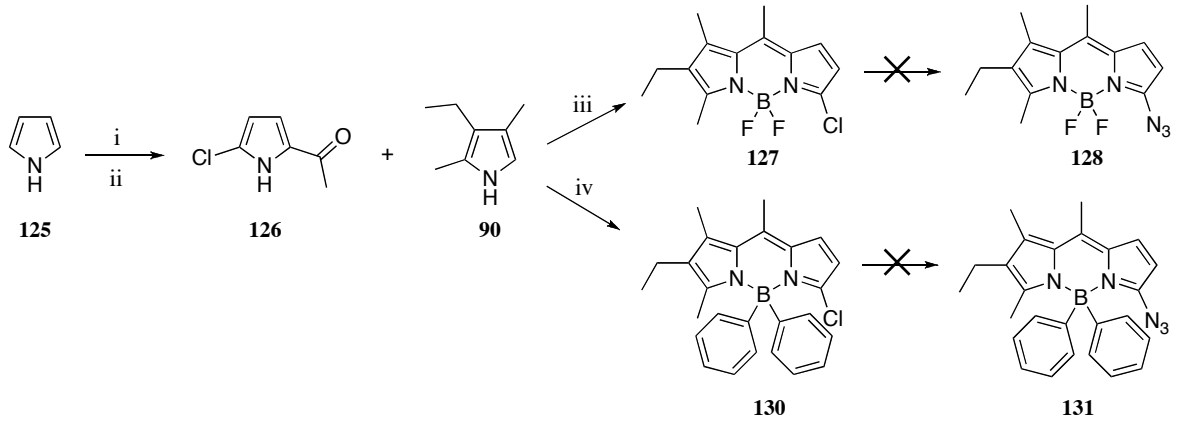


Scheme 3-8 *Regents and conditions*: i) $NaNO_2$, HOAc. 0°C, 3h; ii) NaOMe, Et_2O, 0°C; iii) HOAc, NaOAc, Zn dust, 95-110 °C; iv) , r.t.; v) NaOH, diethylene glycol, 220 °C; vi) NCS, CH_2Cl_2.

2-(Ethoxycarbonyl)-3,4-dimethylpyrrol **121** was prepared from diethyl oximidomalonate **117** and 2-methyl-3-oxa-l-butene 1-oxide sodium salt **120** by Paal-Knorr pyrrole synthesis in the presence of zinc dust (Scheme 3-8).[136] To obtain a more stable dipyrrolemethane intermediate, the pyrrole ester **121** was used to prepare the 5-phenyl-dipyrrolemethane instead of 3,4-dimethylpyrrole. After removal of the ester side chain on the 1,9-positions, chlorination of the 5-phenyl-dipyrrolemethane **123** was attempted by treatment with *N*-chlorosuccinimide (NCS), which, however, failed to give the desired mono-chlorinated product **124**.

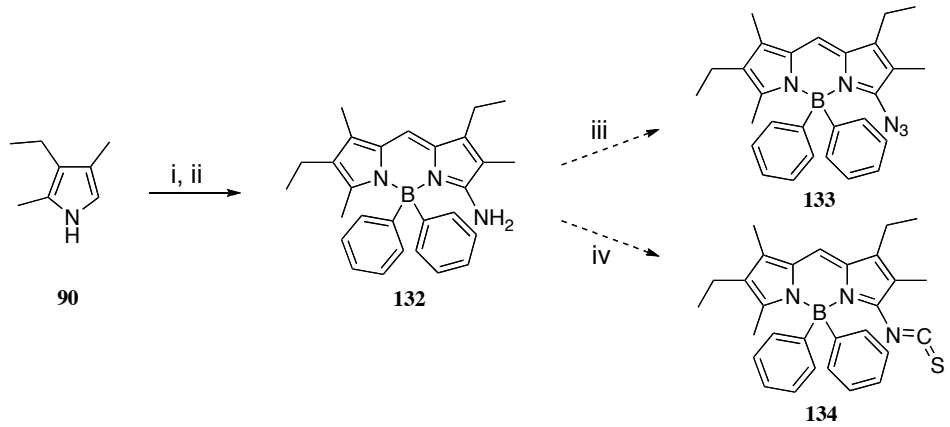
As alternatives, 4,4-difluoro-5-chloro-1,3,8-trimethyl-2-ethyl-4-bora-3a,4a-diaza-*s*-indacene **127** and 4,4-diphenyl-5-chloro-1,3,8-trimethyl-2-ethyl-4-bora-3a,4a-diaza-*s*-indacene **130** were synthesized according to literature procedures (Scheme 3-9).[137] The

monochlorinated BODIPY **127** and **130** were then treated with sodium azide at 60°C, which, however, also failed to give the desired azido-BODIPY products **128** or **131**.



Scheme 3-9 *Regents and conditions*: i) $SOCl_2$, THF. -78 °C, 3h; ii) acylating agent, THF, 14h; iii) $POCl_3$, Et_3N, BF_3, CH_2Cl_2; iv) Et_3N, $BBrPh_2$ (**129**), CH_2Cl_2, r.t.

We next sought to prepare amino-BODIPY as precursor for the synthesis of azido- and isothiocyanato-BODIPY.



Scheme 3-10 *Regents and conditions*: i) $NaNO_2$ (aq) $AcOH/Ac_2O$ (1:1), 100°C, 2h; ii) Et_3N, $BBrPh_2$ (**129**), CH_2Cl_2, rt, 2 h; iii) 2-azido-1,3-dimethylimidazolinium hexafluorophosphate, base, CH_2Cl_2; iv) iv) CS_2, Et_3N, Boc_2O, EtOH. r.t.

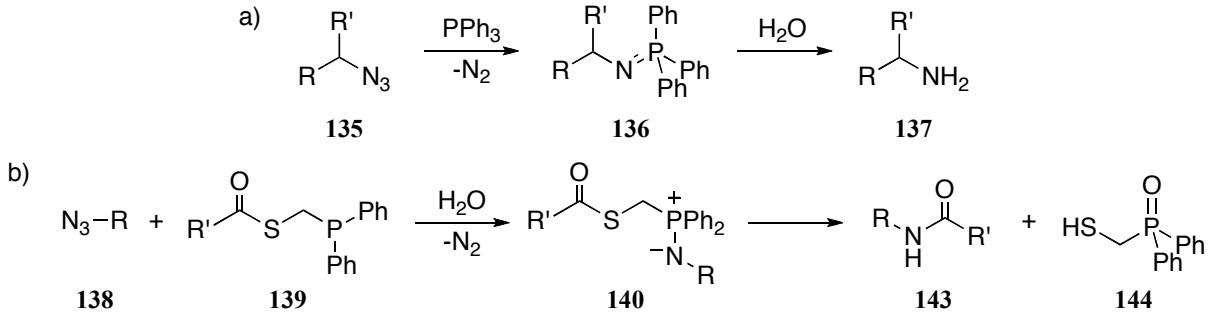
Following a literature procedure,[138] the 4,4-diphenyl analogue **132** was prepared from 2,4-dimethyl-3-ethylpyrrole **90** in a one-pot fashion (Scheme 3-10). The identity of the

product **132** was confirmed by X-ray crystallography. Transformations of **132** into the corresponding azido- and isothiocyanato BODIPY **133** and **134**, respectively, are ongoing in our laboratory.

Chapter 4 Synthesis of PNAs via Staudinger reaction

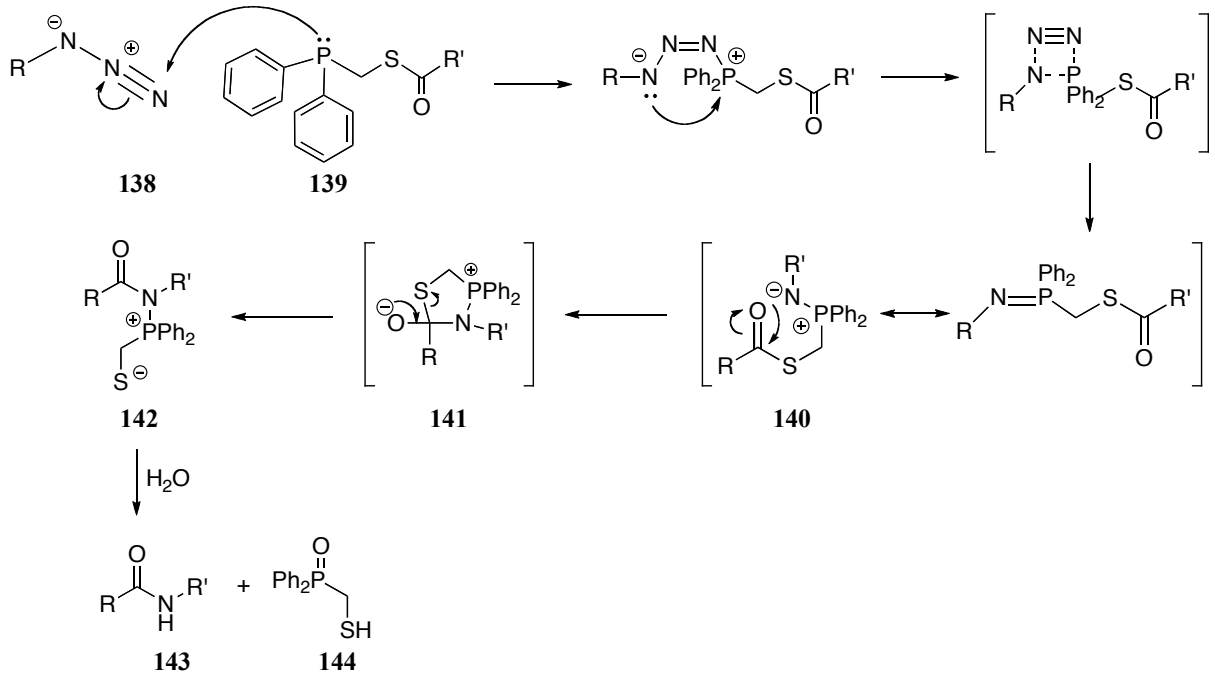
4.1 Overview of Staudinger ligation applied in peptide synthesis

The Staudinger ligation reaction between azides and phosphines (Scheme 4-1b), first demonstrated by Bertozzi and co-workers in 2000,[139] is a modification of the classical Staudinger reaction (Scheme 4-1a) where treatment of azido compound **135** with phosphine leads to the reduction of azide to amine **137**.



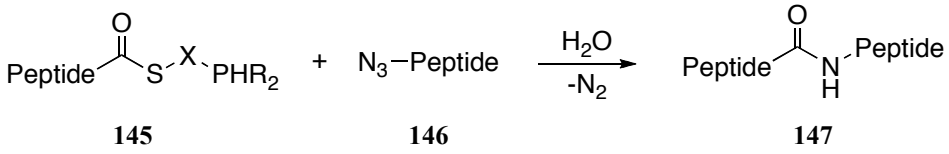
Scheme 4-1. a) Staudinger reaction; b) Staudinger ligation reaction.

The Staudinger ligation occurs between an azide **138** and a thioester of phosphinothiol **139** to produce an aza-ylide intermediate **140**. The nitrogen atom of the iminophosphorane **140** is nucleophilic, which can be acylated by a thioester via formation of a tetrahedral intermediate **141**. In aqueous media, the intermediate rearranges to produce an amidophosphonium salt **142**, which hydrolyzes to give an amide **143** and *o*-(diphenylphosphinyl)-benzenethiol **144**. The Staudinger ligation is therefore a useful approach for chemical ligation. It is noteworthy that the method is "traceless", leaving no residual atoms from the phosphinothiol remaining in the final product.



Scheme 4-2. Proposed mechanism of the Staudinger ligation reaction.

The Staudinger ligation, as a chemoselective ligation chemistry with fewer additives, has been developed for use with biological samples. Nilsson's group reported[140] its application in peptide synthesis, where a peptide with a C-terminal phosphinothioester **145** is coupled to another peptide with an N-terminal α-azido group **146** to generate a single peptide **147** without residual atoms containing (Scheme 4-3).



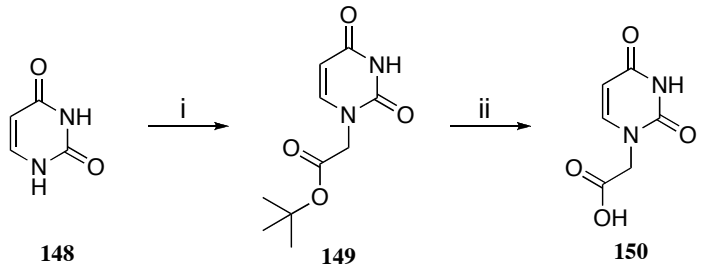
Scheme 4-3. Staudinger ligation in peptide synthesis.

4.2 Synthesis of PNAs by Staudinger ligation reaction

Normally synthesis of PNA oligomers involves coupling the amino group to carboxyl in the presence of activators. These coupling reactions often give rise to products in rather poor

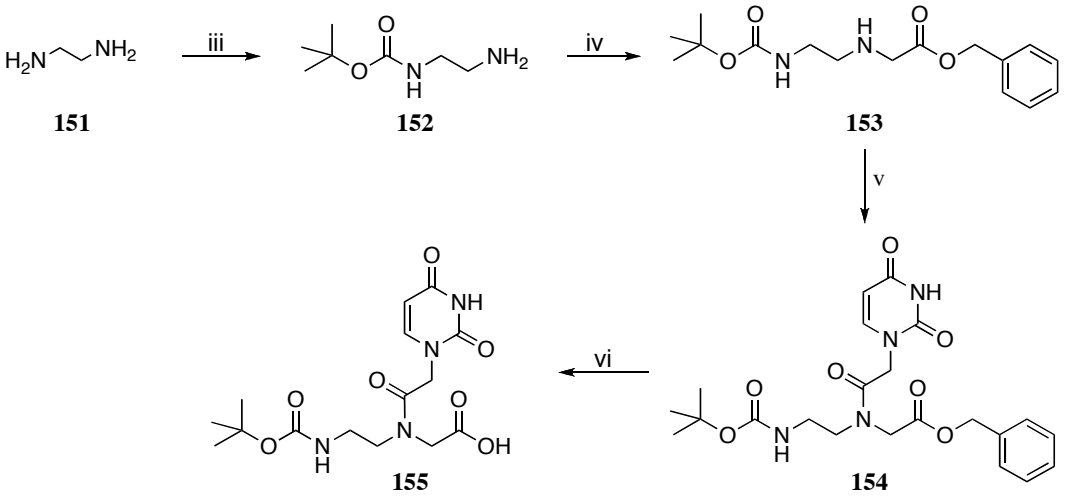
yields, particularly with bases such as guanine. In this thesis the Staudinger ligation was explored for the synthesis of PNA.

As a model study, uracil PNA monomers were prepared. Alkylation of uracil **148** gave the corresponding *tert*-butyl ester **149**, which was subsequently hydrolyzed to give the corresponding acid **150** (Scheme 4-4).



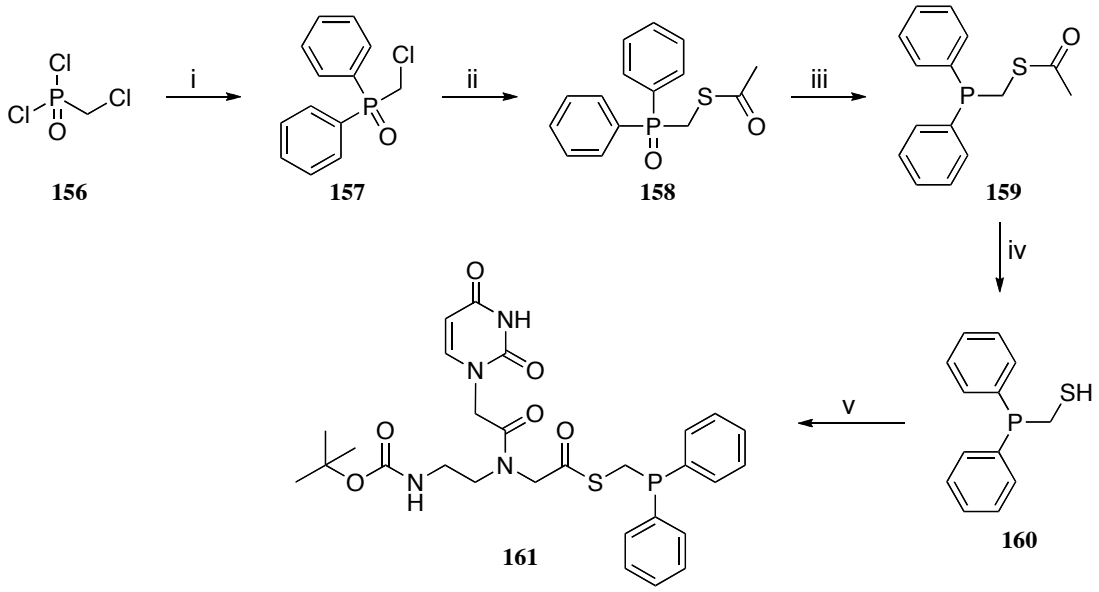
Scheme 4-4. *Reagents and conditions*: i) *tert*-butyl bromoacetate, DMF, K_2CO_3; ii) TFA, CH_2Cl_2

The protected *N*-(2-aminoethyl)glycine unit **153** was prepared from ethylenediamine **151** in two steps (Scheme 4-5). Coupling of the uracil acetic acid **150** with the backbone **151** gave the corresponding fully protected uracil monomer **154**. Deprotection of the benzyl ester gave the corresponding monomer acid **155**.



Scheme 4-5 *Reagents and conditions*: iii) Di-*tert*-butyl dicarbonate, CH_2Cl_2.; iv) benzyl bromoacetate, $(i\text{-}Pr)_2NEt$, 1,4-dioxane; vi) H_2, 10% Pd/charcoal, MeOH-EtOAc (6 mL, 5:1, v/v).

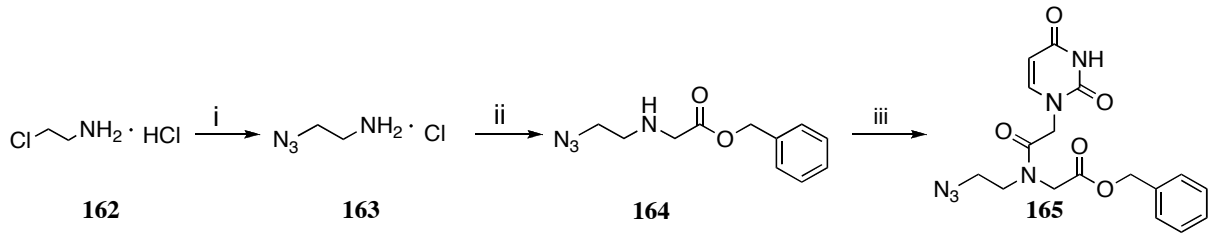
In order to transform monomer acid **155** into corresponding thioester, diphenylmethylphosphinothiol was required. Chloromethylphosphonic dichloride **156** was first treated with phenylmagnesium bromide to generate the phosphine oxide **157** via a Grignard reaction (Scheme 4-6).[140] A mixture of diphenyl chloromethylphosphonate **157**, thioacetic acid and triethylamine was then heated at reflux under nitrogen. The resulting product was purified by chromatography and then treated with decolorizing charcoal to give the thiophosphine oxide **158** in 39% yield. The diphenyl acetylthiomethyl phosphonate **158** was then reduced into phosphinothioester **159** via treatment with an excess of trichlorosilane in chloroform. Hydrolysis of the phosphinoester was performed in methanol with sodium hydroxide under nitrogen, which gave phosphinoester **160** in 30% yield. Coupling of the uracil monomer acid **155** with phosphinoester **160** gave the corresponding uracil-PNA monomer **161**.



Scheme 4-6 *Reagents and conditions*: i) Phenylmagnesium bromide, THF; ii) thioacetic acid, (*i*-Pr)$_2$NEt, THF; iii) trichlorosilane, N_2, $CHCl_3$.; iv) NaOH, N_2, MeOH.; v) **155**, DCC, DMF.

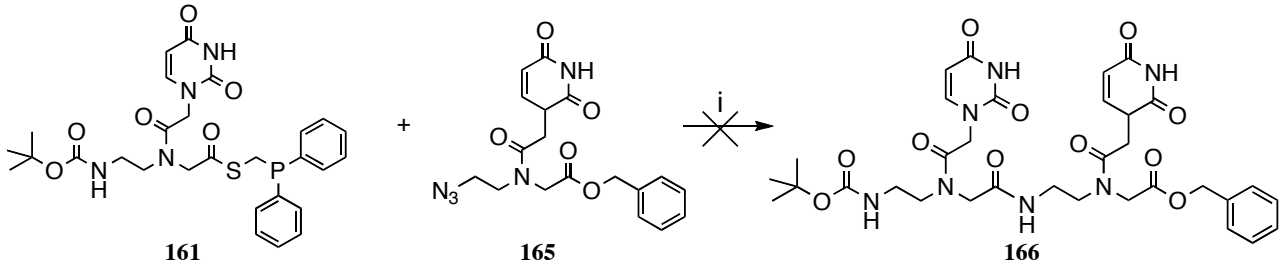
For Staudinger ligation, another uracil building block **165** incorporating an azido group

is required. This azido monomer was prepared by coupling uracil acid **150** with benzyl 2-azidoethylglycine **164**, which was readily prepared in two steps from aminoethyl chloride hydrochloride **162** (Scheme 4-7).



Scheme 4-7 *Reagents and conditions*: i) NaN_3, H_2O, 80°C; ii) benzyl bromoacetate, $(i\text{-Pr})_2$Net, DMF.; iii) **143**, DCC, DMF.

With the azido- **165** and phosphinothioester **161** PNA building blocks ready, ligation reactions were attempted (Scheme 4-8). These reactions have failed to give the desired PNA dimer **166** to date. Work is currently ongoing to further investigate this ligation reaction.



Scheme 4-8 *Reagents and conditions*: i) THF, H_2O, r. t.

Chapter 5 Experimental

5.1 Instrumentation

^{1}H NMR spectra were measured at 300 or 600 MHz with Broker Avance 300 and 600 Digital NMR spectrometers with a 7.05 and 14.1 Tesla Ultrashield magnet, respectively. ^{13}C NMR spectra were measured at 75 MHz or 151 MHz, ^{31}P at 121 MHz, ^{11}B at 96 MHz or 193 MHz, and ^{19}F at 282 MHz, respectively, with the same instrument. Chemical shifts and coupling constants (*J* values) were given in ppm and Hz, respectively. The following deuterated solvents from C/D/N isotopes Inc. were used for preparation of NMR samples: dimethyl-d_6 sulfoxide with 0.05% tetramethylsilane internal standard (99.9 atom % D), deuterated chloroform (99.9 atom % D), and deuterium oxide (99.9 atom % D). Low and high resolution mass spectra were obtained with Kratos Concept 1 S high resolution mass spectrometer using electron impact or fast atom bombardment sources interfaced with DART 32 bit acquisition system through a Sun Sparcstation 10 and Mach 3 software. UV/*vis* spectra were obtained using a Thermospectronic/Unicam UVNis spectrometer configured to the Vision32 software. Fluorescent spectra were recorded using QuantaMaster model QM-2001-4 cuvette-based L-format scanning spectrofluorometer from Photon Technology International (PTI) interfaced with FeliX32 software.

X-Ray crystallography (performed by Dr. Alan Lough at the University of Toronto)

Data were collected on a Bruker-Nonius Kappa-CCD diffractometer using monochromated Mo-Kα radiation and were measured using a combination of ϕ scans and ω scans with κ offsets, to fill the Ewald sphere. The data were processed using the Denzo-SMN

package1. Absorption corrections were carried out using SORTAV2. The structure was solved and refined using SHELXTL V6.13 for full-matrix least-squares refinement that was based on F2. All H atoms were included in calculated positions and allowed to refine in riding-motion approximation with U_{iso} tied to the carrier atom.

5.2 Chromatography

Silica gel (EMD, >230 mesh) was used for short-column chromatography. Thin layer chromatography was performed on Silicycle SiliaPlate F-254 TLC plates using the following solvent mixtures:

System A: hexane–dichloromethane (70:30, v/v)

System B: hexane–dichloromethane (60:40, v/v)

System C: hexane–dichloromethane (50:50, v/v)

System D: hexane–dichloromethane (40:60, v/v)

System E: DCM

System F: Methanol–dichloromethane (5:95, v/v)

System G: methanol–dichloromethane (10:90, v/v)

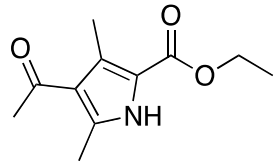
5.3 Solvents and Chemicals

Benzene and diethyl ether were dried by heating under reflux over sodium in the presence of benzophenone for 4 h and then distilled. *N*,*N*-Diethylamine, *N*,*N*-diisopropylamine, piperidine, pyridine and CH_2Cl_2 were dried by heating under reflux over calcium hydride for 4 h and then distilled. Methanol and ethanol were dried by heating over trace amount of iodine activated magnesium with a magnesium loading of 3.0-4.0 g/L. All

other reagents were purchased from Sigma-Aldrich or VWR Canlab without further purification prior to use unless stated otherwise.

5.4 Preparation of compounds

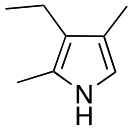
Ethyl 4-acetyl-3,5-dimethyl-1*H*-pyrrole-2-carboxylate 167 [141]



A cold (ice–water bath) solution of sodium nitrite (35.00 g, 0.51 mol) in water (50 mL) was added dropwise over 3 h to a stirred cooled (ice-water bath) mixture of ethyl acetoacetate (43.00 g, 0.33 mol) and acetic acid (150 mL) in a three-neck flask equipped with a thermometer. After the mixture was stirred for another 24 h at room temperature, acetylacetone (37.5 g, 0.038 mol) was added, followed by the addition of zinc dust (48.00 g, 0.74 mol) in portions (3~4 g per portion) at a rate so that the reaction temperature did not exceed 80°C. After the addition of zinc was complete, the mixture was heated at 110°C overnight. The products were poured into ice-water (500 mL) and cooled at -4°C for 2 h. The solid was collected by filtration and further washed with water (3×300 mL). The product was then heated under reflux in 100% ethanol (300 mL) and filtered while hot. The filtrate was allowed to stand at 4°C overnight to give the *title compound* as light yellow crystals (44.20 g, 65%).

δ_H[$CDCl_3$, 300.1 MHz]: 1.39 (3 H, t, J = 7.2), 2.47 (3 H, s), 2.54 (3 H, s), 2.61 (3 H, s), 4.35 (2 H, q, J = 7.2), 9.25 (1 H, br, s)

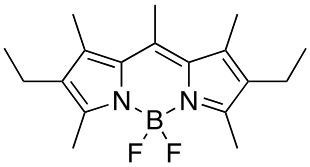
2, 4-Dimethyl-3-ethyl-1*H*-pyrrole 90 [141]



A mixture of ethyl 4-acetyl-3,5-dimethyl-1*H*-pyrrole-2-carboxylate (44.00 g, 0.26 mol) and KOH (35.00 g, 0.89 mol) were suspended in diethylene glycol (250 mL) in a three-neck flask equipped with a thermometer and a condenser. Hydrazine hydrate (14.00 g, 0.28 mol) was added. The mixture was stirred under reflux for 2 h while a steam of nitrogen was bubbled into the reaction mixture. The distillates at 60-100°C were discarded and the residue was heated under reflux for another 1 h. The nitrogen flow was increased and the distillates at 120-200°C were collected. The latter distillate was diluted with water (50 mL) and extracted with ether (2×60 mL). The organic layer was dry ($MgSO_4$) and concentrated under reduced pressure, followed by distillation under vacuum to give the titled product (17.21 g, 67%) as a light yellow oil. This compound should be well desiccated and stored in a refrigerator in dark.

δ_H[$CDCl_3$, 300.1 MHz]: 1.26 (3 H, t, J = 7.4), 2.21 (3 H, s), 2.31 (3 H, s), 2.58 (2 H, q, J = 7.4), 6.51 (1 H, s), 7.52 (1 H, broad s, NH)

4,4-Difluoro-1,3,5,7,8-pentamethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene 92



A solution of distilled acetyl chloride **91** (0.21 mL, 2.9 mmol) in dry dichloromethane (10 mL) was added drop-wise to a stirred degassed solution of 3-ethyl-2,4-dimethylpyrrole **90** (1.10 mL, 8.15 mmol) in dry dichloromethane (15 mL). After the mixture was heated under

reflux for 3 h, the solvents were evaporated under reduced pressure. The residue was co-evaporated with dry toluene (3×20 mL) and then re-dissolved in dry dichloromethane (20.0 mL), followed by addition of dry triethylamine (1.1 mL, 7.9 mmol). After 15 min boron trifluoride diethyl etherate (0.93 mL, 7.4 mmol) was added and the mixture was stirred under reflux for 3 h. Upon cooling to room temperature, the products were extracted successively with water (30 mL) and brine (3×30 mL). The organic layer was separated, dried ($MgSO_4$) and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel. The appropriate fractions, which were eluted with dichloromethane–hexane (50:50 v/v), were combined and evaporated under reduced pressure to give the *title compound* as an orange solid (0.50 g, 54%).

HR-Fab found $[M+H]^+$ =318.19648, $^{12}C_{18}{}^{1}H_{25}{}^{11}B^{19}F_2{}^{14}N_2$ requires 318.20788

R_f (System D): 0.57

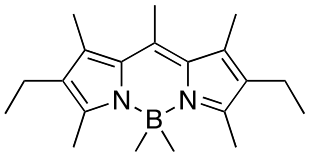
δ_H[$CDCl_3$, 300.1 MHz]: 1.06 (6 H, t, J = 7.5), 2.34 (6 H, s), 2.42 (4 H, q, J = 7.5), 2.52 (6 H, s), 2.61 (3 H, s).

δ_C[$CDCl_3$, 75.5 MHz]: 12.4, 14.4, 14.9, 16.9, 17.1, 131.6, 132.3, 136.3, 139.7, 151.8

δ_B[$CDCl_3$, 192.6 MHz]: 0.66 (t, J = 33)

δ_F[$CDCl_3$, 282.4 MHz]: -146.0 (q, J = 33)

1,3,4,4,5,7,8-Heptamethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene 93[126]



A solution of methylmagnesium bromide in diethyl ether (1.02 mL, 3 *M*, 3.06 mmol) was added to a solution of 4,4-difluoro-1,3,5,7,8-pentamethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene **92** (0.107 g 0.336 mmol) in dry dichloromethane (30 mL) at 0°C. After the mixture was stirred for 5 min (TLC: dichloromethane–hexane, 40:60 v/v), water (2 mL) was added, and the resulting mixture was washed successively with water (30 mL) and brine (30 mL). The layers were separated and the organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel. The appropriate fractions, which were eluted with dichloromethane–hexane (20:80 v/v) were combined and evaporated under reduced pressure to give the *title compound* as an orange solid (0.071 g, 67%).

HR-Fab found $[M+H]^+$ =310.25311, $^{12}C_{20}{}^{1}H_{31}{}^{11}B^{14}N_2$ requires 310.25803

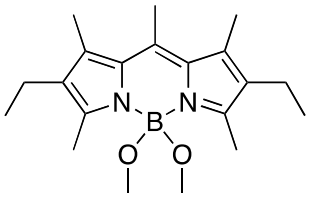
R_f (System A): 0.80

δ_H[$CDCl_3$, 300.1 MHz]: 0.26 (6 H, s), 1.09 (6 H, t, J = 7.5), 2.39 (6 H, s), 2.46 (4 H, q, J = 7.5), 2.48 (6 H, s), 2.69 (3 H, s)

δ_C[$CDCl_3$, 75.5 MHz]: 14.3, 14.8, 15.0, 17.5, 17.6, 53.43, 130.3, 131.9, 132.2, 139.8, 148.7

δ_B[$CDCl_3$, 96.3 MHz]: -1.55

4,4-Dimethoxy-1,3,5,7,8-pentamethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene 94



(Method A: prepared from 4,4-difluoro-1,3,5,7,8-pentamethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene 86)[127]

A solution of sodium methoxide in methanol (0.40 mL, 1.29 mmol, taken from a stock solution prepared by the addition of 0.74 g sodium metal to 10 mL methanol) was added to a solution of 4,4-difluoro-1,3,5,7,8-pentamethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene **92** (0.104 g 0.327 mmol) in dry dichloromethane (30 mL) at 0°C. The mixture was stirred under reflux for 23 h (TLC: dichloromethane–methanol, 92:8 v/v). The resulting mixture was washed successively with distilled water (40 mL) and aqueous saturated sodium bicarbonate solution (30 mL). The layers were separated and the organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel. The appropriate fractions, which were eluted with dichloromethane–methanol (98:2 v/v) were combined and evaporated under reduced pressure to give the *title compound* as an orange solid (0.071 g, 63%).

(Method B: prepared from 4,4-bis(trichloroacetoxy)-1,3,5,7,8-pentamethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene 96)

Dry methanol (0.10 mL) and 1,8-diazabicycloundec-7-ene (DBU, 0.10 mL, 0.67 mmol) were successively added to a solution of 4,4-bis(dichloroacetoxy)-2,6-diethyl-1,3,5,7,8-pentamethyl-4-bora-3a,4a-diaza-*s*-indacene **96** (150 mg, 0.280 mmol) in dry dichloromethane (10 mL). The mixture was stirred for 5 h and the resulting solution was washed successively

with saturated aqueous sodium bicarbonate solution (10 mL) and brine (10 mL). The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel. The appropriate fractions, which were eluted with dichloromethane–methanol (99:1 v/v) were combined and evaporated under reduced pressure to give the *title compound* as an orange solid (87 mg, 91%).

FAB-MS $[M+H]^+$ found 342, $C_{20}H_{31}BN_2O_2$ requires 342.

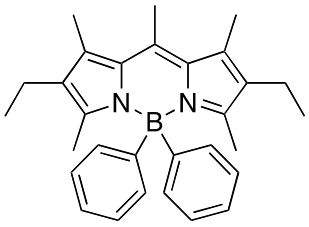
Rf (System D): 0.38

δ_H[$CDCl_3$, 300.1 MHz]: 1.07 (6 H, t, J = 7.5), 2.37 (6 H, s), 2.43 (4 H, q, J = 7.5), 2.50 (6 H, s), 2.63 (3 H, s), 2.82 (6 H, s)

δ_C[$CDCl_3$, 75.5 MHz]: 12.1, 14.6, 15.1, 17.1, 17.2, 49.0, 131.9, 133.1, 134.2, 139.1, 152.0

δ_B[$CDCl_3$, 96.3 MHz]: 2.43

4,4-Diphenyl-1,3,5,7,8-pentamethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene 95 [128]



A solution of phenylmagnesium bromide in diethyl ether (1.02 mL, 3 *M*, 3.06 mol) was added at 0°C to a solution of 4,4-difluoro-1,3,5,7,8-pentamethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene **92** (0.110 g, 0.346 mmol) in dry dichloromethane (30 mL). After the mixture was stirred at 0°C for 10 min (TLC: dichloromethane–hexane, 40:60 v/v), water (2 mL) was added, and the resulting mixture was washed successively with distilled water (30 mL) and brine (30 mL). The organic layer was dried ($MgSO_4$) and concentrated under

reduced pressure. After the products were evaporated under reduced pressure, the residue was purified by column chromatography on silica gel. The appropriate fractions, which were eluted with diethyl ether–hexane (15:85 v/v) were combined and evaporated under reduced pressure to give the *title compound* as an orange solid (0.098 g, 65%).

HR-Fab found $[M+H]^+ = 434.26879$, $^{12}C_{30}{}^{1}H_{35}{}^{11}B^{14}N_2$ requires 434.28933

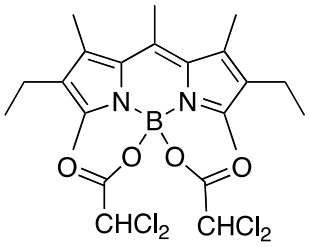
R_f (System A): 0.63

δ_H[$CDCl_3$, 300.1 MHz]: 1.05 (6 H, t, J = 7.5), 1.80 (6 H, s), 2.42 (4 H, q, J = 7.5), 2.44 (6 H, s), 2.74 (3 H, s), 7.28 (10 H, m)

δ_C[$CDCl_3$, 75.5 MHz]: 14.7, 14.8, 15.1, 17.5, 18.0, 125.5, 127.2, 128.1, 132.3, 132.4, 133.5, 133.7, 140.1, 151.2

δ_B[$CDCl_3$, 96.3 MHz]: -0.36

4,4-Bis(trichloroacetoxy)-1,3,5,7,8-pentamethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene 96



Dichloroacetic acid (143 µl, 1.73 mmol) was added to a solution of 4,4-dimethoxy-1,3,5,7,8-pentamethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene **92** (60 mg, 0.175 mmol) in dry dichloromethane (1.5 mL). After 18 h, complete consumption of the starting material was observed by TLC (hexane-dichloromethane, 20:80 v/v). The resulting mixture was diluted

with dichloromethane (10 mL) and washed successively with water (15 mL) and saturated aqueous sodium bicarbonate solution (15 mL). The layers were separated and the organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel. The appropriate fractions, which were eluted with hexane–dichloromethane (60:40 v/v) were combined and evaporated to give the *title compound* as an orange solid (90 mg, 95%).

EI found $M^+ = 536$, $C_{22}H_{27}BCl_4N_2O_4$ requires 536.

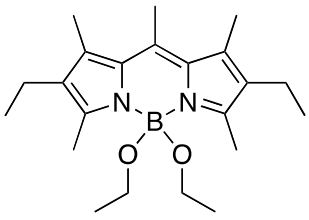
R_f (System F): 0.42

δ_H[$CDCl_3$, 300.1 MHz]: 1.03 (6 H, t, J = 7.6), 2.37 (4 H, q, J = 7.6), 2.37 (6 H, s), 2.44 (6 H, s), 2.70 (3 H, s), 5.88 (2 H, s)

δ_C[$CDCl_3$, 75.5 MHz]: 12.6, 14.7, 17.2, 66.2, 132.8, 133.1, 137.8, 140.8, 150.4, 163.2

δ_B[$CDCl_3$, 96.3 MHz]: 0.54

2,6-Diethyl-4,4-diethoxy-1,3,5,7,8-pentamethyl-4-bora-3a,4a-diaza-*s*-indacene 97



Dry ethanol (0.10 mL) and DBU (0.080 mL, 0.54 mL) were successively added to a solution of 4,4-bis(dichloroacetoxy)-2,6-diethyl-1,3,5,7,8-pentamethyl-4-bora-3a,4a-diaza-*s*-indacene **96** (100 mg, 0.187 mmol) in dry dichloromethane (10 mL). The mixture was stirred for 3 h and the resulting solution was washed successively with saturated aqueous sodium bicarbonate solution (10 mL) and brine (10 mL). The organic layer was dried ($MgSO_4$) and

concentrated under reduced pressure. The residue was purified by column chromatography on silica gel. The appropriate fractions, which were eluted with dichloromethane–methanol (99:1 v/v) were combined and evaporated under reduced pressure to give the *title compound* as an orange solid (52 mg, 75%).

ESI-MS found $[M+Na]^+$ = 393.2. $C_{22}H_{35}BN_2NaO_2^+$ requires 393.27.

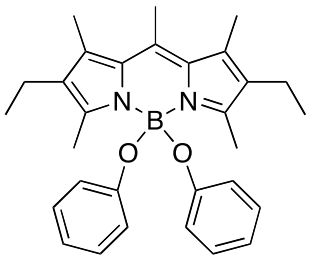
R_f: 0.47 (System F).

$\delta_H[CDCl_3]$: 0.99 (6 H, t, J = 7.0), 1.05 (6 H, t, J = 7.5), 2.35 (6 H, s), 2.42 (4 H, q, J = 7.5), 2.54 (6 H, s), 2.62 (3 H, s), 2.91 (4 H, q, J = 7.0).

$\delta_C[CDCl_3]$: 12.4, 14.5, 15.1, 17.0, 17.2, 17.7, 56.3, 131.8, 133.0, 134.0, 138.9, 152.2.

$\delta_B[CDCl_3]$: -1.76

2,6-Diethyl-4,4-diphenoxy-1,3,5,7,8-pentamethyl-4-bora-3a,4a-diaza-*s*-indacene 98



Phenol (65 mg, 0.69 mmol) and DBU (0.080 mL, 0.54 mmol) were successively added to a solution of 4,4-bis(dichloroacetoxy)-2,6-diethyl-1,3,5,7,8-pentamethyl-4-bora-3a,4a-diaza-*s*-indacene **96** (100 mg, 0.187 mmol) in dry dichloromethane (10 mL). The mixture was stirred for 30 min and the resulting solution was washed successively with saturated aqueous potassium carbonate solution (3×10 mL) and brine (10 mL). The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by column

chromatography on silica gel. The appropriate fractions, which were eluted with dichloromethane–hexane (50:50 v/v) were combined and evaporated under reduced pressure to give the *title compound* as an orange solid (60 mg, 69%).

ESI-MS found $[M+Na]^+ = 489.2$. $C_{30}H_{35}BN_2NaO_2^+$ requires 489.27.

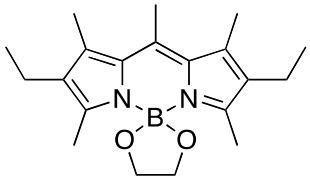
R_f: 0.32 (System C).

$\delta_H[CDCl_3]$: 0.93 (3 H, t, $J = 7.5$), 0.96 (4 H, q, $J = 7.5$), 2.37 (6 H, s), 2.50 (6 H, s), 2.69 (3 H, s), 6.51 (4 H), 6.73 (2 H), 7.02 (4 H).

$\delta_C[CDCl_3]$: 12.7, 14.6, 14.8, 17.0, 17.1, 118.6, 119.1, 128.9, 132.7, 132.9, 136.1, 139.2, 152.7, 156.8.

$\delta_B[CDCl_3]$: 0.67

2-[(*Z*)-(3,5-Dimethyl-4-ethyl-2*H*-pyrrol-2-ylidene)ethyl]-1-(1,3,2-dioxaborolan-2-yl)-3,5-dimethyl-4-ethyl-1*H*-pyrrole 99



To a solution of 4,4-bis(dichloroacetoxy)-2,6-diethyl-1,3,5,7,8-pentamethyl-4-bora-3a,4a-diaza-*s*-indacene **96** (180 mg, 0.336 mmol) in dry dichloromethane (10 mL) was added a solution of ethylene glycol (16 µl, 0.29 mmol) and DBU (0.15 mL, 1.00 mmol) in dichloromethane (3.0 mL). The mixture was stirred for 30 min and the resulting solution was washed successively with saturated aqueous sodium bicarbonate solution (10 mL) and brine (10 mL). The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The

residue was purified by column chromatography on silica gel. The appropriate fractions, which were eluted with dichloromethane–methanol (99:1 v/v) were combined and evaporated under reduced pressure to give the *title compound* as an orange solid (29 mg, 29% based on ethylene glycol).

ESI-MS found $[M+H]^+$ = 341.1. $C_{20}H_{30}BN_2O_2^+$ requires 341.24.

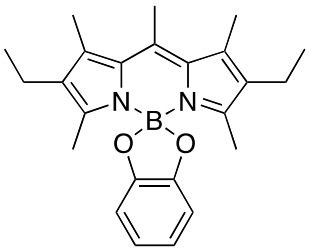
R_f: 0.43 (System F).

$\delta_H[CDCl_3]$: 1.02 (6 H, t, J = 7.5), 2.31 (6 H, s), 2.39 (4 H, q, J = 7.5), 2.48 (6 H, s), 2.60 (3 H, s), 4.18 (4 H, s).

$\delta_C[CDCl_3]$: 12.5, 14.5, 15.0, 17.2, 17.5, 65.2, 132.1, 133.1, 136.1, 139.8, 151.4

$\delta_B[CDCl_3]$: 5.89

1-(1,3,2-Benzodioxaborol-2-yl)-2-[(*Z*)-(3,5-dimethyl-4-ethyl-2*H*-pyrrol-2-ylidene)ethyl]-3,5-dimethyl-4-ethyl-1*H*-pyrrole 100



A solution of catechol (20 mg, 0.18 mmol) and DBU (0.080 mL, 0.54 mmol) in tetrahydrofuran (THF, 3.0 mL) was added slowly to a solution of 4,4-bis(dichloroacetoxy)-2,6-diethyl-1,3,5,7,8-pentamethyl-4-bora-3a,4a-diaza-*s*-indacene **96** (100 mg, 0.187 mmol) in dry THF (5.0 mL). The mixture was stirred for 30 min and the products were concentrated under reduced pressure. The resulting mixture was diluted with dichloromethane (10 mL) and washed successively with saturated aqueous sodium bicarbonate solution (8 mL) and brine (8

mL). The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel. The appropriate fractions, which were eluted with hexane–dichloromethane (90:10 v/v) were combined and evaporated under reduced pressure to give the *title compound* as an orange solid (32 mg, 44%.).

ESI-MS found $[M+H]^+$ = 389.1. $C_{24}H_{30}BN_2O_2^+$ requires 389.24.

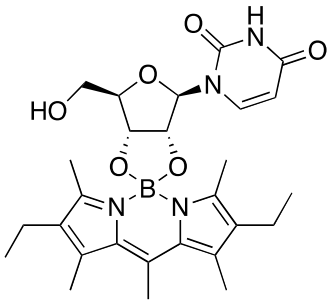
R_f: 0.42 (System C).

$\delta_H[CDCl_3]$: 1.00 (6 H, t, J = 7.5), 2.04, (6 H, s), 2.36 (10 H, br, m), 2.66 (3 H, s), 6.77 (4H, s).

$\delta_C[CDCl_3]$: 12.6, 14.6, 14.9, 17.1, 17.2, 108.7, 119.3, 128.8, 132.5, 133.0, 137.0, 139.6, 152.0, 153.5

$\delta_B[CDCl_3]$: 6.99

Uridine-BODIPY conjugate 101



A solution of uridine (45.0 mg, 0.184 mmol) and 1,8-diazabicycloundec-7-ene (0.080 mL, 0.54 mmol) in DMF (3.0 mL) was added slowly to a solution of 4,4-bis(dichloroacetoxy)-2,6-diethyl-1,3,5,7,8-pentamethyl-4-bora-3a,4a-diaza-*s*-indacene **96** (100 mg, 0.187 mmol) in dry DMF (5.0 mL). After the mixture was stirred for 30 min, the solvent was removed under reduced pressure. The resulting mixture was diluted with dichloromethane (10 mL) and washed successively with saturated aqueous sodium

bicarbonate solution (8 mL) and brine (8 mL). The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel. The appropriate fractions, which were eluted with dichloromethane–methanol (99:1 v/v) were combined and evaporated under reduced pressure to give the uridine-BODIPY conjugate **101** as an orange solid (43 mg, 44%).

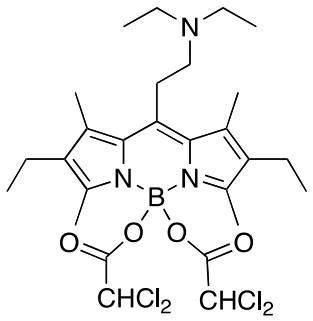
ESI-MS found $[M+H]^+$ = 523.2. $C_{22}H_{36}BN_2NaO_2^+$ requires 523.27.

R_f: 0.27 (System F).

δ_H[DMSO-d_6]: 0.97 (3 H, t, *J* = 7.4), 0.99 (3 H, t, *J* = 7.3), 2.27 (3 H, s), 2.30 (3 H, s), 2.34, 2.37, 2.40 (integrate 7 H), 2.55 (3 H, s), 2.58 (3 H, s), 3.57 (m, 2 H, H-5', H-5''), 4.08 (1 H, dd, *J* = 3.5 and 7.5, H-4'), 4.61–4.68 (2 H, m, H-2' and H-3'), 5.02 (1 H, ex, 5'-OH), 5.61 (1 H, d, *J* = 8.0), 5.92 (1 H, d, *J* = 2.2, H-1'), 7.84 (1 H, d, *J* = 8.0), 11.3 (1 H, s, ex).

δ_H[DMSO-d_6]: 6.90

4,4-Bis(dichloroacetoxy)-2,6-diethyl-8-(2-(*N*,*N*-diethylamino)ethyl)-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene 102



Diethylamine (1.0 mL, 9.7 mmol, used directly from package) was added to a solution of 4,4-bis(dichloroacetoxy)-2,6-diethyl-1,3,5,7,8-pentamethyl-4-bora-3a,4a-diaza-*s*-indacene **96** (100 mg, 0.187 mmol) in dry dichloromethane (5 mL). After the mixture was stirred for 2 d,

the products were diluted with dichloromethane (5 mL) and washed successively with saturated aqueous sodium bicarbonate solution (8 mL) and brine (8 mL). The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel. The appropriate fractions, which were eluted with hexane–dichloromethane (90:10, v/v) were combined and evaporated under reduced pressure to give the *title compound* as an orange solid (48 mg, 41%).

ESI-MS found $[M+H]^+$ = 622.1, $C_{27}H_{39}BCl_4N_3O_4^+$ requires 622.17.

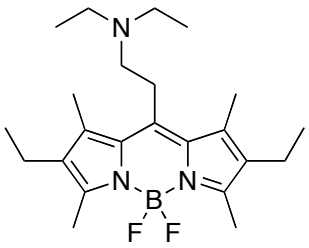
R_f: 0.36 (System F).

$\delta_H[CDCl_3]$: 1.03 (6 H, t, *J* = 7.6), 1.07 (6 H, t, *J* = 7.0), 2.37 (4 H, q, *J* = 7.6), 2.44 (12 H, s), 2.66 (4 H, q, *J* = 7.0), 2.75 (2 H, m), 3.33 (2 H, m), 5.87 (2 H, s).

$\delta_C[CDCl_3]$: 11.5, 12.6, 13.8, 14.6, 17.3, 28.3, 46.5, 54.9, 66.2, 132.7, 133.1, 137.2, 143.4, 150.9, 163.2.

$\delta_B[CDCl_3]$: 0.49

2,6-Diethyl-4,4-difluoro-1,3,5,7-tetramethyl-8-(*N,N*-diethylaminoethyl)-4-bora-3a,4a-diaza-*s*-indacene 104



(Method A: preparation by treating 4,4-difluoro-1,3,5,7,8-pentamethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene 92 with diethylamine)

Diethylamine (0.65 mL, 6.30 mmol) was added to a solution of 4,4-difluoro-1,3,5,7,8-pentamethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene **92** (100 mg, 0.31 mmol) in dry dichloromethane (4 mL) in a Reacti vial. After the mixture was heated at 40˚C for 3 d, the resulting solution was diluted with dichloromethane (10 mL) and washed successively with saturated aqueous ammonium chloride solution (2×10 mL) and brine (10 mL). The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel. The appropriate fractions, which were eluted with dichloromethane–methanol (99:1 v/v) were combined and evaporated under reduced pressure to give the *title compound* as an orange solid (78 mg, 62%).

(Method B: preparation by treating 4,4-difluoro-1,3,5,7,8-pentamethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene 92 with tetraethylmethane diamine)

Tetraethylmethane diamine (0.89 mL, 4.5 mmol) was added to a solution of 4,4-difluoro-1,3,5,7,8-pentamethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene (75 mg, 0.24 mmol) in dry tetrahydrofuran (4 mL) in a Reacti vial. After the mixture was heated at 40˚C overnight, the resulting solution was diluted with dichloromethane (10 mL) and washed successively with saturated aqueous ammonium chloride solution (2×10 mL) and brine (10 mL). The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel. The appropriate fractions, which were eluted with dichloromethane–methanol (99:1 v/v) were combined and evaporated under reduced pressure to give the *title compound* as an orange solid (78 mg, 93%).

R_f: 0.4 (System F).

HR-MS (EI) found: 403.29742, $^{12}C_{23}{}^{1}H_{36}{}^{11}B^{19}F_2{}^{14}N_3$ required: 403.29703

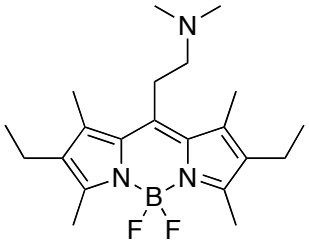
$\delta_H[CDCl_3]$: 1.06 (12 H, t, J = 7.3), 2.38–2.47 (10 H, m), 2.51 (6 H, s), 2.65 (6 H, m), 3.25 (2

H, m).

δ_C[$CDCl_3$]: (75.5 MHz): δ = 11.6, 12.4, 13.5, 14.8, 17.2, 27.8, 46.6, 55.1, 131.2, 132.6, 135.9, 142.3, 152.3.

δ_B[$CDCl_3$]: δ = 0.60 (t, *J* = 33.2)

2,6-Diethyl-4,4-difluoro-1,3,5,7-tetramethyl-8-(*N,N*-dimethylaminoethyl)-4-bora-3a,4a-diaza-*s*-indacene 105



Dimethylamine (1 mL, 2 *M* in THF) was added to a solution of 4,4-difluoro-1,3,5,7,8-pentamethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene **92** (100 mg, 0.31 mmol) in dry dichloromethane (4 mL) in a Reacti vial. After the mixture was heated at 40˚C for 4 d, the resulting solution was diluted with dichloromethane (10 mL) and washed successively with saturated aqueous ammonium chloride solution (2×10 mL) and brine (10 mL). The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel. The appropriate fractions, which were eluted with dichloromethane–methanol (99:1 v/v) were combined and evaporated under reduced pressure to give the *title compound* as an orange solid (72 mg, 61%).

HR-MS (EI) found 375.26626, $^{12}C_{21}{}^{1}H_{32}{}^{11}B^{19}F_2{}^{14}N_3$ required 375.26573.

*R*f: 0.44 (System F)

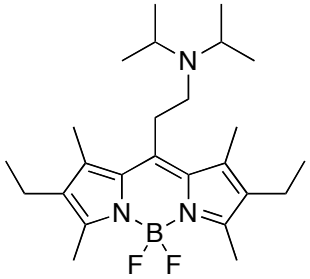
δ_H[$CDCl_3$]: 1.07 (6 H, t, *J* = 7.5), 2.36 (6 H, s), 2.42 (4 H, q, *J* = 7.5), 2.51 (6 H, s), 2.55 (2 H,

m), 3.24 (2 H, m).

$\delta_C[CDCl_3]$: 12.4, 13.5, 14.8, 17.2, 27.3, 45.5, 60.6, 131.2, 132.7, 135.8, 141.6, 152.4.

$\delta_B[CDCl_3]$: 0.59 (t, J = 33.2)

2,6-Diethyl-4,4-difluoro-1,3,5,7-tetramethyl-8-(*N,N*-diisopropylaminoethyl)-4-bora-3a,4a-diaza-*s*-indacene 106



(Method A: reaction was carried out in dichloromethane)

Diisipropylamine (6.73 mL, 47.78 mmol) was added to a solution of 4,4-difluoro-1,3,5,7,8-pentamethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene **92** (300 mg, 0.94 mmol) in dry dichloromethane (7 mL) in a Reacti vial. After the mixture was heated at 40˚C for 7 d, the resulting solution was diluted with dichloromethane (20 mL) and washed successively with saturated aqueous ammonium chloride solution (2×20 mL) and brine (20 mL). The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel. The appropriate fractions, which were eluted with dichloromethane–methanol (99:1 v/v) were combined and evaporated under reduced pressure to recover the starting material (236 mg) and give the *title compound* as an orange solid (30 mg, 34%).

(Method B: reaction was carried out in dibromomethane)

Diisipropylamine (0.89 mL, 6.30 mmol) was added to a solution of 4,4-difluoro-1,3,5,7,8-pentamethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene **92** (100 mg, 0.31 mmol) in dry dibromomethane (4 mL) in a Reacti vial. After the mixture was heated at 60˚C for 24 h, the resulting solution was diluted with dichloromethane (10 mL) and washed successively with saturated aqueous ammonium chloride solution (2×10 mL) and brine (10 mL). The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel. The appropriate fractions, which were eluted with dichloromethane–methanol (99:1 v/v) were combined and evaporated under reduced pressure to give the *title compound* as an orange solid (102 mg, 75%).

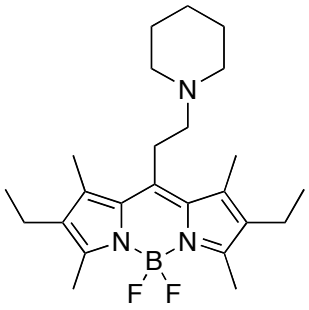
R_f: 0.46. HR-MS (FAB^+) found 432.32985, $[M+H]^+$ required 432.33616.

$\delta_H[CDCl_3]$: 1.02–1.10 (18 H, m), 2.30–2.47 (10 H, m), 2.52 (6 H, s), 2.73 (2 H, m), 3.14 (2 H, sep, J = 6.5), 3.21 (2 H, m).

$\delta_C[CDCl_3]$: 12.4, 13.4, 14.8, 17.2, 20.9, 29.1, 46.9 (2 signals), 131.3, 132.5, 136.0, 143.2, 152.0.

$\delta_B[CDCl_3]$: 0.62 (t, J = 33.2).

2,6-Diethyl-4,4-difluoro-1,3,5,7-tetramethyl-8-((2-piperidin-1-yl)ethyl)-4-bora-3a,4a-diaza-*s*-indacene 107



Piperidine (0.63 mL, 6.39 mmol, 20 mol. equiv.) was added to a solution of 4,4-difluoro-1,3,5,7,8-pentamethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene **86** (100 mg, 0.31 mmol) in dry dichloromethane (4 mL) in a Reacti vial. After the mixture was heated at 40˚C for 4 d, the resulting solution was diluted with dichloromethane (10 mL) and washed successively with saturated aqueous ammonium chloride solution (2×10 mL) and brine (10 mL). The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel. The appropriate fractions, which were eluted with dichloromethane–methanol (99:1 v/v) were combined and evaporated under pressure to give the *title compound* as an orange solid (74 mg, 57%).

HR-MS (EI) found 415.29638, $C_{24}H_{36}BF_2N_3$ required 415.29703.

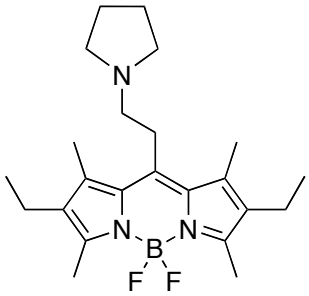
R_f: 0.42. (System F)

$\delta_H[CDCl_3]$: 1.07 (6 H, t, J = 7.5), 1.47 (2 H, m), 1.63 (4 H, m), 2.40 (6 H, s), 2.41 (4 H, q, J = 7.5), 2.51 (10 H, br), 2.59 (2 H, m), 3.26 (2 H, m).

$\delta_C[CDCl_3]$: 12.4, 13.6, 14.8, 17.2, 24.3, 26.0, 26.6, 54.6, 60.5, 131.2, 132.6, 135.8, 142.4, 152.2.

$\delta_B[CDCl_3]$: 0.60 (t, J = 33.6)

2,6-Diethyl-4,4-difluoro-1,3,5,7-tetramethyl-8-((2-pyrrolidin-1-yl)ethyl)-4-bora-3a,4a-diaza-*s*-indacene 108



Pyrrolidine (0.52 mL, 5.27 mmol) was added to a solution of 4,4-difluoro-1,3,5,7,8-pentamethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene **92** (100 mg, 0.31 mmol) in dry dichloromethane (4 mL) in a Reacti vial. After the mixture was heated at 40˚C for 24 h, the resulting solution was diluted with dichloromethane (10 mL) and washed successively with saturated aqueous ammonium chloride solution (2×10 mL) and brine (10 mL). The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel. The appropriate fractions, which were eluted with dichloromethane–methanol (99:1 v/v) were combined and evaporated under reduced pressure to give the *title compound* as an orange solid (80 mg, 63%).

HR-MS (EI) found 401.28081, $^{12}C_{23}{}^{1}H_{34}{}^{11}B^{19}F_2{}^{14}N_3$ required 401.28138.

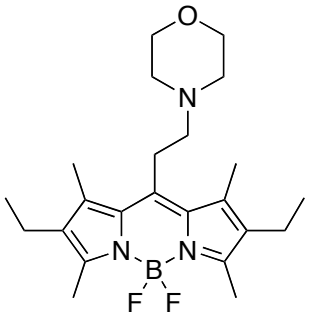
*R*f: 0.36. (System F)

$\delta_H[CDCl_3]$: 1.06 (6 H, t, *J* = 7.5), 1.84 (4 H, m), 2.41 (4 H, q, *J* = 7.5), 2.51 (6 H, s), 2.64 (4 H, m), 2.73 (2H, m), 3.33 (2 H, m).

$\delta_C[CDCl_3]$: 12.4, 13.6, 14.8, 17.2, 23.5, 28.0, 57.4, 131.2, 132.6, 135.8, 141.8, 152.3.

$\delta_B[CDCl_3]$: 0.60 (t, *J* = 33.2)

2,6-Diethyl-4,4-difluoro-1,3,5,7-tetramethyl-8-((2-morpholin-1-yl)ethyl)-4-bora-3a,4a-diaza-*s*-indacene 109



Morpholine (0.52 mL, 6.02 mmol) was added to a solution of 4,4-difluoro-1,3,5,7,8-pentamethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene **92** (100 mg, 0.31 mmol) in dry dibromomethane (4 mL) in a Reacti vial. After the mixture was heated at 60˚C for 7 d, the resulting solution was diluted with dichloromethane (10 mL) and washed successively with saturated aqueous ammonium chloride solution (2×10 mL) and brine (10 mL). The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel. The appropriate fractions, which were eluted with dichloromethane–methanol (99:1 v/v) were combined and evaporated under reduced pressure to recover the starting material (50 mg) and give the *title compound* as an orange solid (48 mg, 66% based on consumed starting material).

HR-MS (FAB^+) found 417.27094, $^{12}C_{23}{}^{1}H_{34}{}^{11}B^{19}F_2{}^{14}N_3$ required 417.27630.

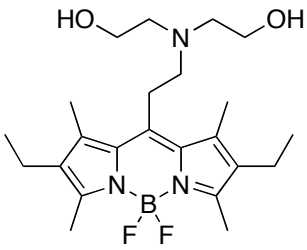
R_f: 0.44 (System F)

$\delta_H[CDCl_3]$: 1.07 (6 H, t, J = 7.6), 2.30–2.46 (10 H, m), 2.51 (6 H, s), 2.54–2.65 (6 H, m), 2.32 (2 H, m), 3.76 (4 H, t, J = 4.4).

$\delta_C[CDCl_3]$: 12.4, 13.6, 14.8, 17.2, 26.3, 53.6, 59.9, 67.0, 131.1, 132.7, 135.7, 141.6, 152.5.

$\delta_B[CDCl_3]$: 0.59 (t, J = 33.2).

8-[*N*,*N*-Bis(hydroxyethyl)]ethyl-4,4-difluoro-1,3,5,7,8-pentamethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene 111



Diethanolamine (330 mg, 3.14 mmol) and *N*,*N*-diisopropylethylamine (0.55 mL, 3.16 mmol) were added to a solution of 4,4-difluoro-1,3,5,7,8-pentamethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene **92** (50 mg, 0.16 mmol) in dioxane–dibromomethane (4 mL, 1:3 v/v) and heated at 60˚C for 7 d in a sealed Reacti vial. Upon cooling, the products were diluted with dichloromethane (10 mL) and washed successively with saturated aqueous ammonium chloride solution (2×10 mL) and brine (10 mL). The layers were separated and the organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel. The appropriate fractions, which were eluted with dichloromethane–methanol (99:1 v/v), were combined and evaporated under reduced pressure to recover unreacted starting material (40 mg) and give the *title compound* as an orange solid (12 mg, 88% based on consumed starting material).

MS-ESI$^+$ found 436.3 for $[M+H]^+$, $C_{23}H_{37}BF_2N_3O_2^+$ required: 436.3.

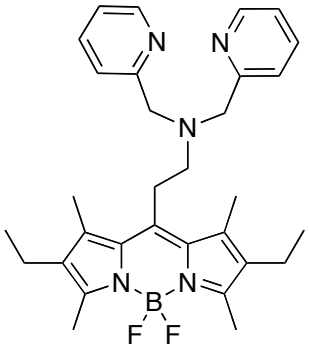
R_f: 0.45 (System F), 0.74 (System G).

$\delta_H(CDCl_3)$: 1.06 (6 H, t, J = 7.5), 2.38 – 2.46 (10 H, s + q, J = 7.5), 2.51 (6 H, s), 2.78 (4 H, t, J = 5.2), 2.82–2.87 (2 H, m), 3.26–3.31 (2 H, m), 3.68 (4 H, t, J = 5.2)

$\delta_C(CDCl_3)$: 12.4, 13.6, 14.8, 17.2, 27.5, 55.8, 56.8, 59.7, 131.1, 132.9, 135.5, 141.2, 152.7.

$\delta_B(CDCl_3)$: 0.54 (t, J = 33).

8-{*N*,*N*-Bis[(pyridine-2-yl)methyl)]}ethyl-4,4-difluoro-1,3,5,7,8-pentamethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene 112



2-Pyridinyl-*N*-(2-pyridinylmethyl)methanamine (312 mg, 1.57 mmmol) and *N*,*N*-diisopropylethylamine (0.27 mL, 1.55 mmol) were added to a solution of 4,4-difluoro-1,3,5,7,8-pentamethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene **92** (50 mg, 0.16 mmol) in dibromomethane (4 mL) and the mixture was heated at 60˚C for 7 d in a sealed React vial. Upon cooling, the products were diluted with dichloromethane (10 mL) and washed successively with saturated aqueous ammonium chloride solution (2×10 mL) and water (10 mL). The layers were separated and the organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel. The appropriate fractions, which were eluted with dichloromethane–methanol (99:1 v/v), were combined and evaporated under reduced pressure to recover unreacted starting material (36 mg) and give the *title compound* as an orange solid (20 mg, 86% based on reacted starting material).

MS-ESI$^+$ found 530.3 $[M+H]^+$, $C_{31}H_{39}BF_2N_5^+$ required: 530.3.

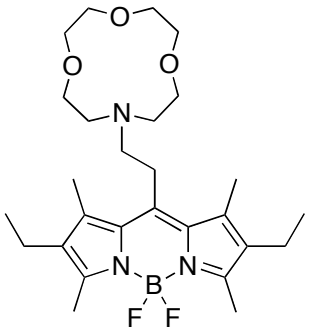
R_f: 0.24 (System F), 0.60 (System G).

$\delta_H(CDCl_3)$: 1.00 (6 H, t, *J* = 7.3), 2.23 (6 H, s), 2.35 (4 H, q, *J* = 7.3), 2.49 (6 H, s), 2.91 (2 H, m, br), 3.26 (2 H, m, br), 3.92 (4 H, s), 7.17 (2 H, t, *J* = 5.5), 7.51 (2 H, d, *J* = 7.5), 7.67 (2 H, t, *J* = 7.5), 8.53 (2 H, d, *J* = 4.8).

$\delta_C(CDCl_3)$: 12.4, 13.4, 14.8, 17.1, 27.3, 56.2, 60.8, 122.2, 123.2, 131.3, 132.6, 135.8, 136.4, 141.8, 149.1, 152.2, 159.3.

$\delta_B(CDCl_3)$: 0.56 (t, *J* = 33.0).

8-[1-(Aza-12-crown-4)]ethyl-4,4-difluoro-1,3,5,7,8-pentamethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene 113



1-Aza-12-crown-4 (110 mg, 0.62 mmol) was added to a solution of 4,4-difluoro-1,3,5,7,8-pentamethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene **92** (100 mg, 0.31 mmol) in dry dibromomethane (4 mL) in a Reacti vial. After the mixture was heated at 60˚C for 48 h, the resulting solution was diluted with dichloromethane (10 mL) and washed successively with saturated aqueous ammonium chloride solution (2×10 mL) and water (3×10 mL). The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel. The appropriate fractions, which were eluted with dichloromethane–methanol (98:2 v/v) were combined and evaporated under reduced pressure to recover the starting material (60 mg) and give the *title compound* as an orange solid (38 mg, 60%).

HRMS (FAB$^+$) found 506.33206 $[M+H]^+$, $C_{27}H_{43}BF_2N_3O_3^+$ required 506.33655.

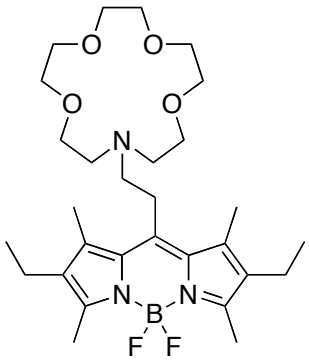
R_f: 0.38 (System F), 0.71 (System G).

$\delta_H(CDCl_3)$: 1.06 (6 H, t, J = 7.6), 2.41 (10 H, s and q, J = 7.5), 2.51 (6 H, s), 2.77–2.85 (6 H, m), 3.26 (2 H, m), 3.71 (12 H, s).

$\delta_C(CDCl_3)$: 12.4, 13.7, 14.8, 17.2, 27.5, 54.9, 58.3, 70.2, 70.4, 71.6, 131.2, 132.7, 135.7, 142.0, 152.3.

$\delta_B(CDCl_3)$: 0.58 (t, J = 33.1).

8-[1-(Aza-15-crown-5)]ethyl-4,4-difluoro-1,3,5,7,8-pentamethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene 114



1-Aza-15-crown-5 (344 mg, 1.57 mmol) and *N*,*N*-diisopropylethylamine (0.27 mL, 1.55 mmol) were added to a solution of 4,4-difluoro-1,3,5,7,8-pentamethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene **92** (50 mg, 0.16 mmol) in dibromomethane (4 mL) and the mixture was heated at 60˚C for 48 h in a sealed Reacti vial. Upon cooling, the products were diluted with dichloromethane (10 mL) and washed successively with saturated aqueous ammonium chloride solution (2×10 mL) and water (3×10 mL). The layers were separated and the organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified

by column chromatography on silica gel. The appropriate fractions, which were eluted with dichloromethane–methanol (99:1 v/v), were combined and evaporated under reduced pressure to give the *title compound* as an orange solid (52 mg, 59%).

HRMS-FAB$^+$ found 550.35764, $C_{29}H_{47}BF_2N_3O_4^+$ required: 550.36277.

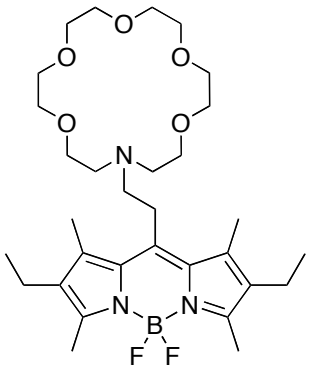
R_f: 0.38 (System F), 0.71 (System G).

$\delta_H(CDCl_3)$: 1.06 (6 H, t, J = 7.5), 2.39 (6 H, s), 2.41 (4 H, q, J = 7.5), 2.50 (6 H, s), 2.79 (2 H, m), 2.87 (4 H, t, J = 6.0), 3.24 (2 H, m), 3.67-3.70 (16 H, m).

$\delta_C(CDCl_3)$: 12.4, 13.6, 14.8, 17.2, 27.8, 54.4, 58.1, 69.9, 70.2, 70.4, 71.1, 131.2, 132.6, 135.8, 141.9, 152.3.

$\delta_B(CDCl_3)$: 0.55 (t, J = 32.2)

8-[1-(Aza-18-crown-6)]ethyl-4,4-difluoro-1,3,5,7,8-pentamethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene 115



1-Aza-18-crown-6 (370 mg, 1.40 mmol) and *N*,*N*-diisopropylethylamine (0.27 mL, 1.55 mmol) were added to a solution of 4,4-difluoro-1,3,5,7,8-pentamethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene **92** (50 mg, 0.16 mmol) in dibromomethane (4 mL) and the mixture was heated at 60˚C for 48 h in a sealed Reacti vial. Upon cooling, the products were diluted

with dichloromethane (10 mL) and washed successively with saturated aqueous ammonium chloride solution (2×10 mL) and water (3×10 mL). The layers were separated and the organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel. The appropriate fractions, which were eluted with dichloromethane–methanol (99:1 v/v), were combined and evaporated under reduced pressure to give the *title compound* as an orange solid (65 mg, 68%).

HRMS (FAB^+) found 594.38334 $[M+H]^+$, $C_{31}H_{51}BF_2N_3O_5^+$ required: 594.38898.

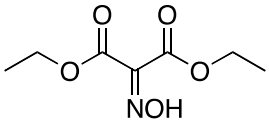
R_f: 0.31 (System F), 0.71 (System G).

$\delta_H(CDCl_3)$: 1.06 (6 H, t, J = 7.5), 2.37 – 2.45 (10 H, s + q, J = 7.5), 2.50 (6 H, s), 2.77–2.82 (2 H, m), 2.87 (4 H, t, J = 5.6), 3.22–3.28 (2 H, m), 3.64–3.68 (20 H, m).

$\delta_C(CDCl_3)$: 12.4, 13.6, 14.8, 17.2, 27.8, 53.9, 57.4, 69.9, 70.4, 70.7, 70.8, 70.9, 131.2, 132.6, 135.8, 142.2, 152.2.

$\delta_B(CDCl_3)$: 0.55 (t, J = 33.0).

Diethyl oximidomalonate 117 [142]

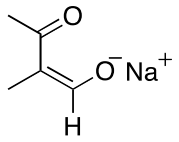


A solution of sodium nitrite (51.00 g, 0.72 mol) in water (50 mL) was added dropwise over a period of 4 h to a mixture of diethyl malonate (40.00 g, 0.25 mol) and glacial acetic acid (120 mL) at 0°C. The reaction mixture was then allowed to warm up to room temperature and stirred for 24 h. The products were diluted with diethyl ether (2×100 mL) and the organic layer was washed first with water (150 mL), then with saturated aqueous sodium bicarbonate

until the washings were alkaline, and finally washed again with water (150 mL). The organic layer was dried ($MgSO_4$) and evaporated under reduced pressure to give the *title compound* as a colorless oil (44.10 g, 90%).

$\delta_H(CDCl_3)$: 1.35 (6 H, dt, J = 7.2 and 13.8), 4.39 (4 H, dq, J = 7.8 and 13,8), 10.22 (1H, br, s, OH).

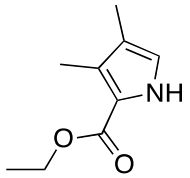
2-Methyl-3-oxa-l-butene 1-oxide sodium salt 120[136]



Sodium metal (7.30 g, 0.32 mol) was added with precaution to methanol (50 mL) at 0 ^oC. After the sodium dissolved, the mixture was heated under reflux for 0.5 h. The resulting sodium methoxide/methanol solution was then diluted with diethyl ether (350 mL) and cooled (ice–water bath). To this mixture was then added over a period of 1 h a mixture of 2-butanone (22.80 g, 0.32 mol) and ethyl formate (23.40 g, 0.32 mol) while the reaction temperature was kept at 4–6°C. After the addition was complete, stirring was allowed to continue, first at 4–6°C for 30 min, and then overnight at room temperature. The resulting colorless powder was filtered to give the titled salt (23.50 g, 61%).

$\delta_H(CDCl_3)$: 1.62 (s, 3H), 2.13 (s, 3H), 8.99 (s, 1H).

2-(Ethoxycarbonyl)-3,4-dimethylpyrrole 121 [136]

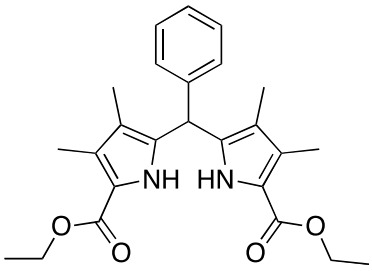


Glacial acetic acid (150 mL) was placed in a 1-L round-bottom flask and heated to 85 ^{o}C, followed by sequential addition of sodium acetate (30.50 g, 0.36 mol), 2-methyl-3-oxa-l-butene 1-oxide sodium salt **120** (35.4 g, 0.29 mol), diethyl oximidomalonate **117** (52.8 g, 0.28 mol), and then a solution of acetic acid (70 mL) in water (30 mL). After addition was completed, the reaction mixture was heated to 95^{o}C followed by addition of zinc dust (45.00 g, 0.69 mol) over 45 min while maintaining the reaction temperature at 95–110^{o}C. After the addition was complete, the reaction mixture was stirred at the same temperature for additional 45 min. The hot products were directly poured into ice-water mixture (700 mL). The solid was collected by filtration and washed with water. The product was then heated in 100% ethanol (400 mL) under reflux and filtered while hot to remove the excessive zinc. The filtrate was concentrated under reduced pressure to remove the solvents. The crude material was purified by column chromatography on silica. The appropriate fractions, which were eluted with dichloromethane, were combined and evaporated under reduced pressure to yield the *title compound* as a light yellow solid (8.60 g, 18%).

R_f: 0.51 (System E)

$\delta_H(CDCl_3)$: 1.37 (3 H, t, J = 7.2), 2.03 (3 H, s), 2.30 (3 H, s), 4.27 (2 H, q, J = 7.2), 6.67 (1 H, d, J = 2.0), 8.85 (1 H, br, s).

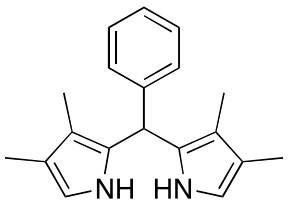
5-Phenyl-2,3,7,8-tetramethyldipyrromethane-1,9-dicarboxylate 122 [143]



Boron trifluoride diethyl etherate (0.3 mL, 2.4 mmol) was added to a solution of ethyl 3,4-dimethyl-2-pyrrolecarboxylate (2.00 g, 12.0 mmol) and benzaldehyde (0.60 mL, 5.9 mmol) in dichloromethane (30 mL). The mixture was stirred under nitrogen for 24 h at room temperature and the products were then washed with saturated aqueous sodium bicarbonate solution (3×30 mL). The layers were separated and the aqueous layer was back extracted with dichloromethane (2×30 mL). The combined organic layers were further washed with brine (2×30 mL), dried ($MgSO_4$) and then evaporated to dryness to give *title compound* as a yellow solid (1.85 g, 75%).

$\delta_H(CDCl_3)$: 1.34 (3 H, t, J = 7.2), 1.80 (3H, s), 2.27 (3 H, s), 4.27 (2 H, q, J = 7.2), 5.50 (1 H, s), 7.12 (2 H, d, J = 7.8), 7.31-7.38 (3 H, m), 8.22 (2 H, br, s).

5-Phenyl-2,3,7,8-tetramethyldipyrromethane 123 [143]

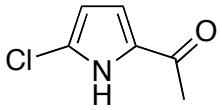


5-Phenyl-2,3,7,8-tetramethyldipyrromethane-1,9-dicarboxylate **122** (300 mg, 0.71 mmol) was dissolved in ethylene glycol (5 mL) containing sodium hydroxide (200 mg, 2.4 mmol), and the solution was heated at 220^{o}C for 1 h. Upon cooling, the reaction mixture was diluted

with brine (15 mL) and extracted with dichloromethane (3×15 mL). The combined organic layers were washed with brine (15 mL), dried ($MgSO_4$) and evaporated to dryness. The residue was further dried *in vaccuo* to give 5-phenyl-2,3,7,8-tetramethylpyrromethane as a black oil (170 mg, 81%).

$\delta_H(CDCl_3)$: 1.84 (12 H, s), 2.06 (12 H, s), 5.52 (1 H, s), 6.41 (2 H, d, J = 1.2), 7.17 (2 H, d, J = 8.0), 7.27-7.37 (3 H, m).

2-Acetyl-5-chloropyrrole 126 [137]



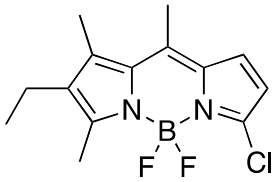
Sulfuryl chloride (0.75 mL, 9.3mmol) was added dropwise to a stirred solution of pyrrole (0.65 mL, 9.3 mmol) in anhydrous tetrahydrofuran (50 mL) in a nitrogen atmosphere at -78 °C. The resulting solution was stirred at -78 °C for 3 h. In a separate flask, the acylating agent was prepared for the Vilsmeier reaction by mixing dimethylamide (0.67 mL, 10 mmol) and $POCl_3$ (0.93 mL, 10 mmol) at 0°C. The mixture was stirred at room temperature until the Vilsmeier reagent salt was formed. Dichloromethane (25 mL) was added to the Vilsmeier reagent and then the solution was added dropwise over 10 min to the solution that contained the pyrrole substrate. The resulting solution was stirred at room temperature for 14 h and then heated under reflux for 15 min. An aqueous solution of sodium carbonate (50 mL, 0.5 *M*) was added and the reaction mixture was heated under reflux for additional 30 min. Upon cooling, the products were extracted with diethyl ether (3×50 mL). The combined organic layers were washed with saturated aqueous sodium bicarbonate solution (2×100 mL) and

water (2×150 mL), dried ($MgSO_4$) and evaporated under reduced pressure to dryness. The residue was purified by column chromatography on silica gel. The appropriate fractions, which were eluted with dichloromethane–hexane (30:70 v/v), were pooled and evaporated under reduced pressure to yield the target pyrrole derivative as a colorless solid (2.12 g, 74%).

R_f: 0.31 (System E)

$\delta_H(CDCl_3)$: 2.41 (3 H, s), 6.15 (1 H, dd, J = 2.7, 3.9), 6.84 (1 H, dd, J = 2.7, 3.9), 9.25 (1 H, br, s).

3-Chloro-4,4-difluoro-6-ethyl-5,7,8-trimethyl-4-bora-3a,4a-diaza-*s*-indacene 127 [137]



To a solution of 2-acetyl-5-chloropyrrole **126** (325 mg, 2.3 mmol) in dichloromethane (10 mL) under nitrogen was added 3-ethyl-2,4-dimethylpyrrole **90** (310 μL, 2.3 mmol, 1 mol. equiv.) and the resulting solution was cooled (ice-water bath), followed by the addition of $POCl_3$ (220 μL, 2.3 mmol, 1 mol. equiv.). After the reaction mixture was stirred at room temperature for 6 h (during this period the mixture became dark), triethylamine (3.2 mL, 23 mmol) was added and the mixture was stirred for 10 min. Upon cooling (ice-water bath), boron trifluoride diethyl etherate (3.1 mL, 25 mmol) was added dropwise and the reaction mixture was stirred at room temperature for 1 h. The orange solution was diluted with diethyl ether (200 mL) and extracted with water (3×100 mL). The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was then purified by column chromatography on silica gel. The appropriate fractions, which were eluted with

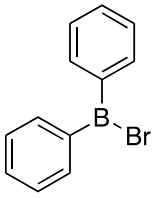
dichloromethane–hexane (70:30 v/v), were combined and evaporated under reduced pressure to give the *title compound* as an orange solid (500 mg, 74%).

R_f: 0.49 (System C)

$\delta_H(CDCl_3)$: 1.02 (3 H, t, J = 7.5), 1.78 (3 H, s), 3.92 (2 H, q, J = 7.5), 2.44 (3 H, s) 2.64 (3 H, s), 6.22 (1 H, d, J = 5.2), 7.05 (1 H, s, J = 2.7),

$\delta_B(CDCl_3)$: 0.58 (t, J = 33)

Diphenyl-boronbromide 129 [144]

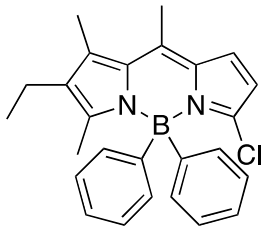


Tetraphenyltin (10.0 g, 23.4 mmol) was placed in a flask and cooled to -70^{o}C, followed by dropwise addition of boron tribromide (4.4 mL, 46.8 mmol) over 20 min while the temperature was maintained at -70 ^{o}C. After addition is complete, the reaction mixture was allowed to warm up to 0^{o}C over a period of about 1 h. Stirring was continued for additional 2 h and the reaction mixture was heated at 220^{o}C for another 2 h. The mixture was then distilled to give the *title compound* as a light yellow oil (125-130 ^{o}C/2 mmHg 7.00 g, 61%).

$\delta_H(CDCl_3)$: 0.93 (3 H, t, J = 7.5), 1.09 (3 H, t, J = 7.5), 1.50 (3 H, s) 7.39-7.48 (10 H, m).

$\delta_B(CDCl_3)$: 66.4

3-Chloro-4,4-diphenyl-6-ethyl-5,7,8-trimethyl-4-bora-3a,4a-diaza-*s*-indacene 130



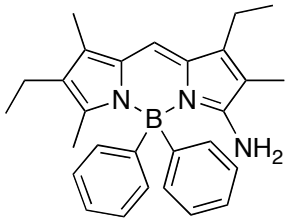
To a solution of 2-acetyl-5-chloropyrrole **126** (400 mg, 2.8 mmol) in dichloromethane (8 mL) under an atmosphere of nitrogen was added 2,4-dimethylpyrrole **90** (380 μL, 2.8 mmol, 1 mol equiv.) and the resulting solution was cooled (ice-water bath), followed by addition of $POCl_3$ (260 μL, 2.8 mmol, 1 mol. equiv.). After the solution was stirred at room temperature for 6 h (during this period the mixture became dark), triethylamine (1 mL, 7.2 mmol) was added and the mixture was stirred for 10 min. Diphenyl boronbromide (1.35 g, 5.5 mmol) was then added dropwise while the reaction mixture was cooled (ice-water bath). After the reaction mixture was stirred at room temperature for 1 h, the orange products were poured into diethyl ether (200 mL) and extracted with water (3×100 mL). The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The product was purified by flash column chromatography on silica gel. The appropriate fractions, which were eluted with dichloromethane–hexane (30:70 v/v), were combined and evaporated under reduced pressure to give the *title compound* as an orange solid (780 mg, 68%).

R_f: 0.68 (System B)

$\delta_H(CDCl_3)$: 1.02 (3 H, t, J = 7.5), 1.78 (3 H, s), 3.92 (2 H, q, J = 7.5), 2.44 (3 H, s) 2.64 (3 H, s), 6.22 (1 H, d, J = 5.2), 7.05 (1 H, s, J = 2.7), 7.17-7.41 (10 H, m)

$\delta_B(CDCl_3)$: 0.43

4,4-Difluoro-4-bora-3a,4a-diaza-3-amino-1,6-diethyl-3,5,7-trimethyl-*s*-indacene 132 [138]



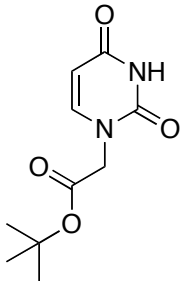
A solution of sodium nitrite (180 mg, 2.6 mmol) in water (2 mL) was added dropwise to another solution of 2,4-dimethyl-3-ethylpyrrole (1 mL, 7.4 mmol) in acetic acid (30 mL) and acetic anhydride (30 mL), and the mixture was heated at 100 ºC for 2 h. The solvents were then removed under reduced pressure. The result product was diluted with dichloromethane (40 mL) and washed with saturated aqueous sodium bicarbonate solution (2×40 mL). The organic phase was dried ($MgSO_4$) and evaporated to dryness under reduced pressure. The residue was dissolved in dichloromethane (40 mL) and triethylamine (2.8 mL, 20 mmol) followed after 30 min diphenyl boron bromide **129** (7.30 g, 30 mmol) were added. After 2 h, the reaction mixture was washed with water (3×40 mL), dried ($MgSO_4$) and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel. The appropriate fractions, which were eluted with dichloromethane–hexane (3:7 v/v), were pooled and concentrated under reduced pressure to give the *title compound* as a orange solid (230 mg, 15%).

R_f: 0.52 (System B)

$\delta_H(CDCl_3)$: 0.93 (3 H, t, J = 7.5), 1.09 (3 H, t, J = 7.5), 1.50 (3 H, s) 7.39-7.48 (10 H, m).

$\delta_B(CDCl_3)$: -0.50

***tert*-Butyl uracil-1-yl acetate 149** [145]

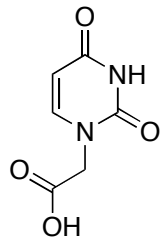


To a suspension of dried uracil (1.50 g, 13.5 mmol) and K_2CO_3 (1.90 g, 13.9 mmol) in DMF (20 mL) was added *tert*-butyl bromoacetate (2.1 mL, 17.8 mmol) at 0°C. The mixture was stirred for 15 h at room temperature and then filtered. The filtrate was evaporated under reduced pressure and the residue was diluted with water (60 mL) and extracted with ethyl acetate (3×50 mL). The combined organic phases were washed with water (50 mL), dried ($MgSO_4$), and then evaporated under reduced pressure. The residue was purified by column chromatography on silica gel. The appropriate fractions, which were eluted with dichloromethane–hexane (6:4 v/v), were combined and evaporated under reduced pressure to afford the *title compound* (2.10 g, 69%) as a white amorphous powder.

R_f: 2.8 (System E)

δ_H(DMSO): 1.51 (9 H, s), 4.37 (2 H, s), 5.76 (1 H, d, J = 7.8), 7.12 (1 H, d, J = 7.8); 8.45 (1 H, br, s)

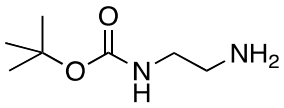
Uracil-1-yl acetic acid 150 [145]



tert-Butyl uracil-1-yl acetate **149** (2.00 g, 8.9 mmol) was dissolved in TFA–CH_2Cl_2 (30 mL, 1:1 v/v) and the mixture was stirred at room temperature for 3 h. The solvents were evaporated under reduced pressure and the crude residue was triturated with diethyl ether. The amorphous solid was filtered off to afford the *title compound* (1.40 g, 93%).

δ_H(DMSO-d_6): 3.65 (1 H, br, s), 4.41 (2 H, s), 5.59 (1 H, dd, J = 1.8 and 7.8), 7.61 (1H, d, J = 7.8), 11.34 (1 H, s).

***N*-Boc-ethylenediamine 152** [146]

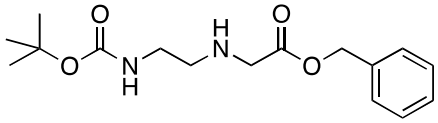


A solution of di-*tert*-butyl dicarbonate (2.12 g, 9.7 mmol) in dry dichloromethane (120 mL) was added dropwise to a solution of ethylenediamine (6.7 mL, 0.1mol) in dichloromethane (250 mL) over a period of 6 h with vigorous stirring and cooling (ice-water bath). After the reaction mixture was stirred for additional 24 h at room temperature, the products were concentrated to *ca*. 150 mL. The resulting mixture was washed with aqueous sodium carbonate (3×50 mL). The organic phase was dried ($MgSO_4$) and evaporated to dryness under reduced pressure and then further dried *in vaccuo*. The residue was purified by column chromatography on silica gel. The appropriate fractions, which were eluted with

concentrated aqueous ammonium hydroxide–methanol–chloroform (1:4:10, v/v), were combined and evaporated under reduced pressure to obtain the *title compound* as a colorless oil (1.45 g, 93%).

$\delta_H(CDCl_3)$: 1.37 (9 H, s), 1.59 (2 H, s), 2.72 (2 H, t, J = 6.4), 3.10 (2 H, q, J = 7.0), 5.12 (1 H, br, s).

_N_-Benzyl-_N'_-Boc-ethylenediamine 153 [147]

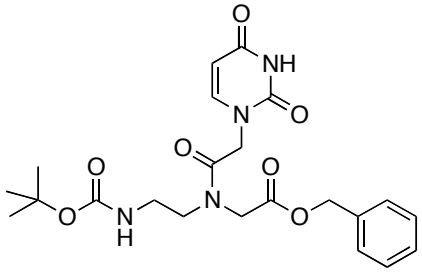


To a solution of *N*-Boc-ethylenediamine **145** (1.45 g, 9.0 mmol) in 1,4-dioxane (10 mL) at 0°C was added a solution of benzyl bromoacetate (1.43 mL, 0.9 mol) in 1,4-dioxane (20 mL) over a period of 1 h, and the reaction mixture was stirred overnight. The solvent was evaporated under reduced pressure, and the residue was dissolved in water (50 mL) and extracted with ethyl acetate (3×50 mL). The combined organic phase was dried over $MgSO_4$, and the solvent was removed under reduced pressure. The crude residue was purified by chromatography with dichloromethane–methanol (98:2, v/v) to afford the desired compound (2.10 g, 76%) as a colorless oil.

R_f: 0.47 (System E)

$\delta_H(CDCl_3)$: 1.45 (9 H, s); 1.70 (1 H, br, s), 2.75 (2 H, t, J = 5.7), 3.20 (2 H, q, J = 5.4), 3.40 (2 H, s), 5.06 (1 H, br, s), 5,17 (2 H, s), 7.36 (5 H, m).

Benzyl *N*-[2-(*N*-Boc-amino)ethyl]-*N*-(uracil-1-acetyl)glycinate 154

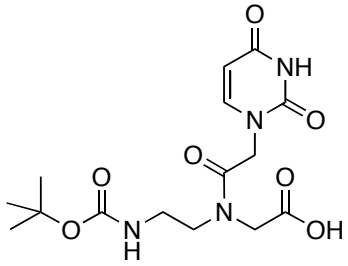


N-Benzyl-*N*-Boc-ethylenediamine **153** (145 mg, 0.47 mmol) and uracil-1-yl acetic acid **150** (88 mg, 0.52 mmol) were dissolved in DMF (8 mL) followed by addition of dicyclohexylcarbodiimide (120 mg, 0.58 mmol) and 4-dimethylaminopyridine (10 mg, 0.08 mmol). The mixture was stirred for 12 h at room temperature and the precipitate was removed by filtration. The filtrate was concentrated under reduced pressure, and the residue was purified by column chromatography on silica gel. The appropriate fractions, which were eluted with dichloromethane–methanol (99:1, v/v), were pooled and evaporated under reduced pressure to give the *title compound* as a white solid (125 mg, 72%).

R_f: 0.50 (System E)

δ_H(DMSO-d_6): 1.37 (2.25, s, rotamer 1), 1.38 (6.75 H, s, rotamer 2), 3.03 (0.5 H, q, J = 6.0, rotamer 1), 3.17 (1.5 H, q, J = 6.0, rotamer 2), 3.33 (0.5 H, t, J = 6.6, rotamer 1), 3.42 (1.5 H, t, J = 6.6, rotamer 2), 4.12 (1.5 H, s, rotamer 2), 4.38 (0.5 H, s, rotamer 1), 4.54 (0.5 H, s, rotamer 1), 4.72 (1.5 H, s, rotamer 2), 5.13 (1.5 H, s, rotamer 2), 5.21 (0.5 H, s, rotamer 1), 5.57 (0.25 H, dd, J = 1.8 and 3.9, rotamer 1), 5.59 (0.75 H, dd, J = 1.8 and 7.8, rotamer 2), 6.77 (0.25 H, t, J = 5.4, rotamer 1), 6.96 (0.75 H, t, J = 5.4, rotamer 2), 7.34-7.42 (6 H, m), 11.32 (0.25 H, s, rotamer 1), 11.32 (0.75 H, s, rotamer 2).

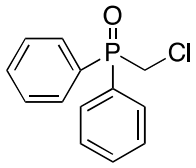
***N*-[2-(*N*-Boc-amino)ethyl]-*N*-(uracil-1-acetyl)glycine 155**



Benzyl *N*-[2-(*N*-Boc-amino)ethyl]-*N*-(uracil-1-acetyl)glycinate **154** (100 mg, 0.27 mmol) was dissolved in methanol–ethyl acetate (6 mL, 5:1 v/v) followed by addition of 10% Pd on charcoal (7 mg). The reaction mixture was stirred in an atmosphere of H_2 for 3 h at room temperature and then filtered through a pad of Celite. The filtrate was concentrated to give the *title compound* as a white solid in virtually quantitative yield (80 mg).

$\delta_H(D_2O)$: 1.35 (3.6 H, s, rotamer 1), 1.36 (5.4 H, s, rotamer 2), 3.19 (0.8 H, t, J = 6.0, rotamer 1), 3.31 (1.2 H, t, J = 6.0, rotamer 2), 3.45 (0.8 H, t, J = 6.0, rotamer 1), 3.50 (1.2 H, t, J = 6.0, rotamer 2), 4.01 (0.8 H, s, rotamer 1), 4.06 (1.2 H, s, rotamer 2), 4.59 (0.8 H, s, rotamer 1), 4.76 (1.2 H, s, rotamer 2), 5.8 (1 H, d, J = 7.8, rotamer 2), 7,47 (1 H, dd, J = 5.4 and 7.8)

Diphenyl chloromethylphosphonate 157 [140]



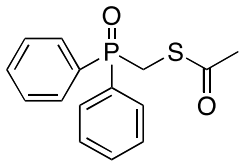
Chloromethylphosphonic dichloride **156** (3.00 g, 18.0 mmol) was dissolved in freshly distilled THF (40 mL) followed by dropwise addition of a solution of phenylmagnesium bromide in THF (36 mL, 1.0 *M*, 36 mmol) over 1 h. The resulting mixture was heated under reflux for 24 h. Upon cooling, the reaction was quenched by the addition of water (3 mL), and

volatiles were removed under reduced pressure. The residue was taken up in dichloromethane and washed with water (15 mL) and brine (15 mL). The organic layer was dried ($MgSO_4$) and evaporated under reduced pressure. The residue was purified by flash column chromatography, eluted with dichloromethane–methanol (99:1, v/v) to give the *title compound* as a white solid (2.57 g, 57%).

$\delta_H(CDCl_3)$: 4.03 (2 H, d, J = 6.3), 7.43-7.53 (6 H, m), 7.72-7.79 (4 H, m).

$\delta_P(CDCl_3)$: 28.2.

Diphenyl acetylthiomethylphosphonate 158 [140]



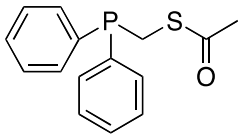
Diphenyl chloromethylphosphonate **157** (2.00 g, 8.0 mmol) was dissolved in THF (10 mL) followed by addition of thioacetic acid (3.6 mL, 51.2 mmol). The resulting solution was cooled (ice-water bath) followed by dropwise addition of diisopropylethylamine (8.9 mL, 51.2 mmol). The reaction mixture was then heated under reflux for 24 h while a stream of nitrogen gas is bubbled into the reaction mixture. Upon cooling, the volatiles were removed under reduced pressure. The resulting black oil was dissolved in dichloromethane (30 mL) and washed successively with hydrochloric acid (2 *N*, 15 mL), saturated aqueous sodium bicarbonate solution (15 L) and brine (15 mL). The organic layer was dried ($MgSO_4$) and filtered. Activated charcoal was added to this solution, which was then heated under reflux for 30 min. The activated charcoal was removed by filtration, and the filtrate was concentrated under reduced pressure, and the residue was purified by flash column chromatography, eluted

with dichloromethane–hexane (70:30 v/v), to give the title thioacetate (0.92 mg, 39%) as orange oil.

$\delta_H(CDCl_3)$: 2.24 (3 H, s), 3.75 (2 H, d, J = 8.4), 7.43-7.53 (6 H, m), 7.72-7.79 (4 H, m).

$\delta_P(CDCl_3)$: 28.9

Acetylthiomethyl diphenylphosphine 159 [140]

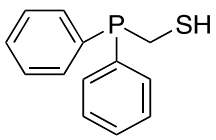


Diphenyl acetylthiomethyl phosphonate **158** (0.92 g, 3.2 mmol) was dissolved in anhydrous chloroform (10 mL) followed by addition of trichlorosilane (4.3 mL, 42.7 mmol). After the reaction mixture was stirred in an atmosphere of nitrogen for 72 h, the products were concentrated under reduced pressure. The residue was purified by flash chromatography, eluted with dichloromethane–hexane (30:70 v/v), to give the *title compound* as a black oil (0.42 g, 48%).

$\delta_H(CDCl_3)$: 2.31 (3 H, s), 3.41 (2 H, d, J = 3.6), 7.34-7.72 (10 H, m).

$\delta_P(CDCl_3)$: -15.7

(Diphenylphosphino)methanethiol 160 [140]



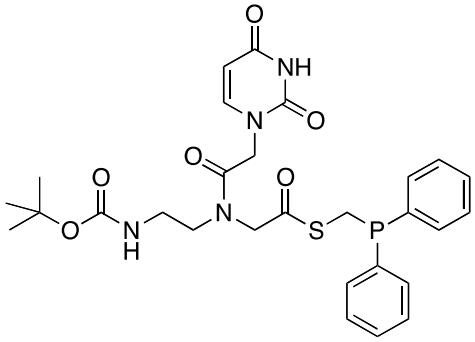
Acetylthiomethyl diphenylphosphine **159** (420 mg, 1.5 mmol) was dissolved in anhydrous methanol (15 mL), and argon gas was bubbled through the solution for 1 h. Sodium hydroxide

(180 mg, 3.0 mmol) was added, and the mixture was stirred under nitrogen for 2 h. Solvents were removed under reduced pressure, and the residue was taken up in dichloromethane (10 mL) and washed with hydrochloric acid (2 *N*, 2×10 mL) and brine (10 mL). The organic layer was dried ($MgSO_4$) and evaporated under reduced pressure. The residue was purified by flash chromatography, eluted with dichloromethane, to give the *title compound* as a clear oil (106 mg, 30%).

$\delta_H(CDCl_3)$: 1.40 (1 H, q, J = 7.5), 3.41 (2 H, dd, J = 2.7 and 8.1), 7.39-7.48 (10 H, m).

$\delta_P(CDCl_3)$: -7.9.

Uracil-PNA monomer 161



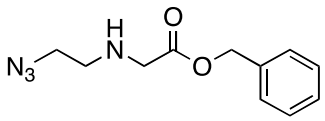
Phosphinothiol **160** (106 mg, 0.46 mmol) and *N*-[2-(Boc-amino)ethyl]-*N*-(uracil-1-acetyl)glycine **155** (185 mg, 0.50 mmol) were dissolved in DMF (10 mL) under an atmosphere of nitrogen, followed by addition of dicyclohexylcarbodiimide (103 mg, 0.50 mmol). After the reaction mixture was stirred for 12 h at room temperature, the precipitate was removed by filtration. The filtrate was concentrated under reduced pressure, and the residue was purified by column chromatography on silica gel, eluted with dicloromethane–methanol (99:1 v/v), to give the titled product as a white solid (110 mg, 42%).

R_f: 0.58 (System E)

$\delta_H(CDCl_3)$: 1.45 (3.2, s, rotamer 1), 1.46 (5.8 H, s, rotamer 2), 3.23 (2 H, m), 3.48 (2 H, m), 3.56 (1.3 H, d, *J* = 4.2, rotamer 2), 3.61 (0.7 H, d, *J* = 4.3, rotamer 1), 4.20 (1.3 H, s, rotamer 2), 4.25 (0.7 H, s, rotamer 1), 4.27 (0.7 H, s, rotamer 1), 4.59 (1.3 H, s, rotamer 2), 4.93 (0.35 H, t, *J* = 6.0, rotamer 1), 5.35 (0.65 H, t, *J* = 6.0, rotamer 2), 5.72 (2 H, d, *J* = 7.6), 7.00 (0.3 H, d, *J* = 7.8, rotamer 2), 7.11 (0.7 H, d, *J* = 7.8, rotamer 2), 7.34-7.46 (10 H, m), 8.72 (0.75 H, s, rotamer 2), 8.77 (0.35 H, s, rotamer 1).

$\delta_P(CDCl_3)$: -13.9 (0.35 P, rotamer 1), -14.9 (0.65 P, rotamer 2)

Benzyl 2-azidoethylglycine 164

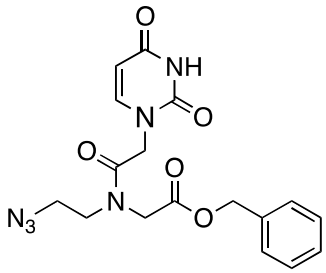


To a solution of 2-aminoethyl chloride hydrochloride salt (1.00 g, 8.7 mmol) in water (10 mL) was added sodium azide (1.70 g, 25.8 mmol) and the reaction mixture was heated at 80^{o}C for 15 h. The solution was concentrated under reduced pressure and then further dried under high vacuum. The residue was then co-evaporated with toluene (2×8 mL) and dry toluene (5 mL). The resulting solid was suspended in DMF (20 mL), followed first by addition of the Hunig's base (3.0 mL, 16.5 mmol) and then dropwise addition of benzyl 2-bromoacetate (1.1 mL, 7.0 mmol). The reaction mixture was stirred for 6 h at 60^{o}C and then diluted with water (50 mL) and extracted with ethyl acetate (3×50 mL). The organic phase was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by flash chromatography, eluted with dicloromethane–methanol (99:1 v/v), to give the *title compound* (0.87 mg, 53%) as a light yellow oil.

R_f: 0.4 (System E)

δ_H(DMSO): 2.31 (1 H, br, s), 2.75 (2 H, t, *J* = 6.0), 3.32 (2 H, t, *J* = 6.0), 3.44 (2 H, s), 5.13 (2 H, s), 7.30-7.40 (m, 5 H, Ar).

U-PNA monomer (2) 165



Benzyl 2-azidoethylglycine **164** (220 mg, 0.94 mmol) and uracil-1-yl acetic acid **150** (112 mg, 1.0 mmol) were dissolved in DMF (3 mL) followed by addition of dicyclohexylcarbodiimide (206 mg, 1.0 mmol) and DMAP (12 mg, 0.10 mmol). After the reaction mixture was stirred for 12 h at room temperature, the precipitate was removed by filtration. The filtrate was concentrated under reduced pressure, and the residue was purified by column chromatography on silica gel. The appropriate fractions, which were eluted with dichloromethane–methanol (99:1 v/v), were combined and evaporated to get the *title compound* (472 mg, 54%) as a white solid.

R_f: 0.63 (System E)

δ_H(DMSO): 3.50 (2 H, m), 3.58 (2 H, m), 4.16 (1.1 H, s, rotamer 2), 4.30 (0.9 H, s, rotamer 1), 4.46 (0.9 H, s, rotamer 1), 4.70 (1.1 H, s, rotamer 2), 5.16 (1.1 H, s, rotamer 2), 5.23 (0.9 H, s, rotamer 1), 5.70 (0.45 H, d, J = 7.8, rotamer 1), 5.71 (0.55 H, d, *J* = 7.8, rotamer 2), 7.08 (0.45 H, d, *J* = 7.8, rotamer 2), 7.18 (0.55 H, d, *J* = 7.8, rotamer 2), 7.31-7.37 (5 H, m), 9.95 (1 H, br, s).

Reference

(1) Lacowicz, J. R. *Principles of Fluorescence Spectroscopy***,** *3rd ed*.; Springer: New York, **2006**, 2

(2) Noël, B.; Volker, L.; Wim, D. *Chem. Soc. Rev*. **2012**, *41*, 1130

(3) (a) Su, X.; Xiao, X. J.; Zhang, C.; Zhao, M. P. *Appl. Spectrosc*. **2012**, *66*, 1249; (b) Sekar, R. B.; Periasamy, A. *J. Cell. Biol*., **2003**, *160*, 629; (c) Kobayashi, H.; Ogawa, M.; Alford, R.; Choyke, P. L.; Urano, Y. *Chem. Rev.* **2010**, *110*, 2620; (d) King, W.; Proffitt, J.; Morrison, L. *Mol. Diagn*. **2000**, *5*, 309

(4) (a)Ventral, M.; Zhai, D. T.; Er, J. C.; Chang, Y. T. *Chem. Rev*., **2012**, *112*, 4391; (b) Kohl, T; Haustein, E; Schwille, P. *Biophys. J.* **2005**, *89*, 2770

(5) (a) Perlette, J.; Tan, W. H. *Anal. Chem*. **2001**, *73*, 5544; (b) Kues, T.; Peters, R.; Kubitscheck, U. *Biophys. J*. **2001**, *80*, 2954

(6) Treibs, A.; Kreuzer, F. *Just. Lieb. Ann. Chem*. **1968**, *718*, 208

(7) Wories, H. J.; Koek, J. H.; Lodder, G.; Lugtenburg, J.; Fokkens, R.; Driessen, O.; Mohn, G. R. *Recu. Trav. Chim. Pays-Bas*. **1985**, *104*, 288

(8) Falk, H.; Hofer, O.; Lehner, H. *Monatsh. Chem.* **1974**, *105*, 169

(9) Monsma, F.; Barton, A.; Kang, H.; Brassard, D.; Haugland, R.; Sibley, D. *J. Neurochem.* **1989**, *52*, 1641

(10) Ulrich, G.; Ziessel, R.; Harriman, A. *Angew. Chem. Int. Ed*. **2008**, *47*, 1184

(11) Loudet A, Burgess K. *Chem. Rev*. **2007**, *107*, 4891

(12) Haugland, R. P. Ed. The Molecular Probes® handbook, a guide to fluorescent probes and labeling technologies (11th ed.); Invitrogen Corp., Carlsbad, CA, **2005**, pp. 57-921

(13) Hinkeldey, B,; Schmitt, A,; Jung, G, *Chemphyschem* **2008**, *9*, 2019

(14) (a) Arroyo, I.; Hu, R.; Merino, G.; Tang, B.; Pena-Cabrera, E. *J. Org. Chem.* **2009**, *74*, 5719; (b) Tram, K.; Yan, H.; Jenkins, H.; Vassiliev, S.; Brucec, D. *Dyes Pigments* **2009**, *82*, 392; (c) Schmitt, A.; Hinkeldey, B.; Wild, M.; Jung, G. *J. Fluoresc.* **2009**, *19*, 755

(15) (a) Qin, W. W; Baruah, M.; Van der, Auweraer, M.; De Schryver, F. C.; Boens, N. *J. Phys. Chem. A* **2005**, *109*, 7371; (b) Ulrich, G.; Goze, C.; Guardigli, M.; Roda, A.; Ziessel, R. *Angew. Chem. Int. Ed*. **2005**, *44*, 3694; (c) Zhu, S. L.; Zhang, J. T.; Vegesna,

G.; Luo, F. T.; Green, S. A.; Liu, H. Y. *Org. Lett*. **2011**, *13*, 438; (d) Niu, S. L.; Ulrich, G.; Ziessel, R.; Kiss, A.; Renard, P. Y.; Romieu, A. *Org. Lett.* **2009**, *11*, 2049

(16) (a) Shah, M.; Thangaraj, K.; Soong, M.-L.; Wolford, L. T.; Boyer, J. H.; Politzer, I. R.; Pavlopoulos, T. G. *Heteroatom. Chem*. **1990**, *1*, 389; (b) Boyer, J. H.; Haag, A. M.; Sathyamoorthi, G.; Soong, M. L.; Thangaraj, K.; Pavlopoulos, T. G. *Heteroat. Chem*. **1993**, *4*, 39

(17) Wang, D.; Fan, J.; Gao, X.; Wang, B.; Sun, S.; Peng, X. *J. Org. Chem.* **2009**, *74*, 7675

(18) Wu, L.; Burgess, K. *Chem. Commun*. **2008**, *40*, 4933

(19) Wood, T. E.; Thompson, A. *Chem. Rev.* **2007**, *107*, 1831

(20) (a) Nijegorodov, N. I.; Downey, W. S. *J. Phys. Chem.* **1994**, *98*, 5639; (b) McFarland, S. A.; Finney, N. S. *J. Am. Chem. Soc*. **2001**, *123*, 1260; (c) Wardle, B. Principles and Applications of Photochemistry (1st ed.); John Wiley & Sons, Ltd., Hoboken, New Jersey, 2009, pp. 66-68

(21) (a) Wada, M.; Ito, S et al. *Tetrahedron Lett.* **2001**, *42*, 6711; (b) Shen, Z; Röhr, H.; Rurack, K.; Uno, H.; Spieles, M.; Schulz, B.; Reck, G.; Ono. *Chem. Eur. J.* **2004**, *10*, 4853

(22) (a) Qin, W.; Rohand, T.; Dehaem, W.; Clifford, J. N.; Driesen, K.; Beljonne, D.; Averbeke, B. V.; Auweraer, M. V.; Boens, N. *J. Phys. Chem. A* **2007**, *111*, 8588; (b) Thoresen, L. H.; Kim, H.; Welch, M. B.; Burghart, A.; Burgess, K. *Synlett* 1**998**, *11*, 1276; c) Thoresen, L. H.; Kim, H.; Welch, M. B.; Burghart, A.; Burgess, K. *Synlett*. **1998**, 1276; d) Burghart, A.; Kim, H.; Welch, M. B.; Thoresen, L. H.; Reibenspies, J.; Burgess, K.; Bergström, F.; Johansson, L. B. A. *J. Org. Chem*. **1999**, *64*, 7813; (e) Baruah, M.; Qin, W.; Flors, C.; Hofkens, J.; Vallee, R. A. L.; Beljonne, D.; Van, der Auweraer, M.; De Borggraeve, W. M.; Boens, N. *J. Phys. Chem*. *A* **2006**, *110*, 5998; (f) Rurack, K.; Kollmannsberger, M.; Daub, J. *Angew. Chem. Int. Ed*. **2001**, *40*, 385

(23) Okujima, T.; Tomimori, Y.; Nakamura, J.; Yamada, H.; Uno, H.; Ono, U. *Tetrahedron* **2010**, *66*, 6895

(24) (a) Kang, H. C.; Haugland, R. P., US. Pat, 5433896, **1995**; (b) Vasilenko, N. P.; Mikhailenko, F. A.; Rozhinskii, Y. I. *Dyes Pigments* **1981**, *2*, 231

(25) Jiong, C.; Armin, B.; Agnes, D. K.; Kevin, B. *J. Org. Chem*. **2000**, *65*, 2900

(26) Kim, H.; Burghart, A.; Welch, M.B.; Reibenspies J.; Burgess, K. *Chem. Commun*. **1999**, *18*, 1889

(27) (a) Murase, S.; Tominaga, T.; Kohama, A. Eur. Pat. 1253151a, **2002**; (b) Ziessel, R.; Ulrich, G.; Harriman, A. *New J. Chem.* **2007,** *31*, 496; Ulrich, G.; Goze, C.; Guardigli, M.; Roda, A.; Ziessel, R. *Angew. Chem. Int. Ed*. **2005**, *44*, 3694

(28) Kee, H. L.; Kirmaier, C.; Yu, L.; Thamyongkit, P.; Youngblood, W. J.; Calder, M. E.; Ramos, L.; Noll, B. C.; Bocian, D. F.; Scheidt, W. R.; Birge, R. R.; Lindsey, J. S.; Holten, D. *J. Phys. Chem. B.* **2005**, *109*, 20433

(29) Tahtaoui, C.; Thomas, C.; Rohmer, F.; Klotz, P.; Duportail, K.; Mély, Y.; Bonnet, D.; Hibert, M. *J. Org. Chem.* **2007**, *72*, 269

(30) Sayre, L. M.; Perry, G.; Smith, M. A. *Curr. Opin. Chem. Biol*. **1999**, *3*, 220

(31) (a) Cohem, A. R.; Trotzky, M. S.; Pincus, D. *Pediatrics*, **1981**, *67*, 904; (b) Tong, S.; von Schirnding, Y. E.; Prapamontol, T. *Bull. World Health Organ.* **2000**, *78*, 1068; (c) Flegal, A. R.; Smith, D. R. *Environ. Res*. **1992**, *58*, 125; (d) Bridges, C. C.; Zalups, R. K. *Toxicol. Appl. Pharmacol*. **2005**, *204***,** 274; (e) Bolger, P. M.; Schwetz, B. A. *New Engl. J. Med*. **2002**, *347*, 1735; (f) Godwin, H. A. *Curr. Opin. Chem. Biol.* **2001**, *5*, 223; (g) White, L. D.; Cory-Slechta, D. A.; Gilbert, M. E.; Tiffany-Castiglioni, E.; Zawia, N. H.; Virgolini, M.; Rossi-George, A.; Lasley, S. M.; Qian, Y. C.; Basha, M. R. *Toxicol. Appl. Pharmacol*. **2007**, *225*, 1

(32) (a) Miller, J. R.; Rowland, J.; Lechler, P. J; Desilets, M.; Hsu, L. C. *Water Air Soil Pollut.* **1996,** *86*, 373; (b) Harris, H. H.; Pichering, I. J. ; George, G. N. *Science* **2003**, *301*, 1203

(33) (a) Xiang, Y.; Tong, A.; Lu, Y. *J. Am. Chem. Soc*., **2009**, *131*,15352; (b) Dean, K. M.; Qin, Y; Palmer, A. E. *Biochim. Biophys. Acta, Mol. Cell. Res*. **2012**, *1823*, 1406; (c) Valeur, B.; Leray, I. *Coord. Chem. Rev*. **2000**, *205*, 3; (d) Jiang, P. J.; Guo, Z. J. *Coord. Chem. Rev*. **2004**, *248*, 205

(34) (a) Tian, M.; Feng, F.; Meng, S.; Yuan, Y. *Chin. Chem. Lett*. **2009**, *20*, 326; (b) Park, J.; Kim, Y. *Analyst*. **2012**, *137*, 3246.; (c) Qi, X.; Jun, E. J.; Xu, L.; Kim, S. J.; Joong Hong, J. S.; Yoon, Y. J.; Yoon. J. *J. Org. Chem*. **2006**, *71*, 2881

(35) (a) Coskun, A.; Engin, U. A. *J. Am. Chem. Soc*. **2005**, *127*, 10464; (b) Zhang, X.; Xu, Y.; Guo, P.; Qian, X. *New J. Chem*. **2012**, *36*, 1621

(36) (a) Vedamalai, M.; Wu, S. *Org. Biomol. Chem*. **2012**, *10*, 5410; (b) Kim, D.; Yamamoto, K.; Ahn, K. H. *Tetrahedron* **2012**, *68*, 5279; (c) Moon, S. Y.; Cha, N. R.; Kim, Y. H.; Chang, S. *J. Org. Chem.* **2004**, *69*, 181; (d) Knut, R.; Matthias, K.; Ute, R. G.; Jörg, D. *J. Am. Chem. Soc.* **2000**, *122*, 968

(37) (a) Cheng, T.; Xu, Y.; Zhang, S.; Zhu, W.; Qian, X.; Duan, L. *J. Am. Chem. Soc*. **2008**, *130*, 16160; (b) Cheng, T.; Wang, T.; Zhu, W.; Yang, Y.; Zeng, B.; Xu, Y.; Qian, X. *Chem. Commun*. **2011**, *47*, 3915

(38) (a) Blaustein, M. P. *Am. J. Physiol. Cell. Physiol.* **1977**, *232*, 165; (b) Huffman, D. L.; O'Halloran, T. V. *Annu. Rev. Biochem*. **2001**, *70*, 677; (c) Aisen, P.; Listowsky, I. *Annu. Rev. Biochem*. **1980**, *49*, 357; (d) Nilius, B,; Droogmans, G. *Physiol. Rev.* **2001**,

81, 1415; (e) Schroeder, J. I. *Plant. Mol. Biol.*, **1995**, 2*8*, 353; (f) Hoffmann, E. K., *Philos Trans R Soc London [Biol],* **1982**, 299, 519

(39) (a) Yamada, K.; Nomura, Y.; Citterio, D.; Iwasawa, N.; Suzuki, K. *J. Am. Chem. Soc*. **2005**, *127*, 6956; (b) Martin, V. V.; Rothe, A.; Gee, K. R. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1851

(40) (a) Malval, J. P.; Leray, I.; Valeurab, B. *New J. Chem*. **2005**, *29*, 1089.; (b) Hirata, T.; Terai, T.; Komatsu, T.; Hanaoka, K.; Nagano, T. *Bioorg. Med. Chem. Lett*. **2011**, *21*, 6090; (c) Namkung, W.; Padmawar, P.; Mills, A. D.; Verkman, A. S. *J. Am. Chem. Soc*. **2008**, *130*, 7794; (d) Baruah, M.; Qin, W.; Vallée, R. A. L.; Beljonne, D.; Rohand, T.; Dehaen, W.; and Boens, N. *Org. Lett.* **2005**, *7*, 4377

(41) Cha, N. R.; Moon, S. Y.; Chang, S. K. *Tetrahedron. Lett.* **2003**, *44*, 8265

(42) Fan, J.; Liu, X.; Hu, M.; Zhu, H.; Song, F.; Peng, X. *Analytica. Chim.. Acta*. **2012**, *735*, 107

(43) (a) Wu, Y.; Peng,X.; Guo, B.; Fan, J.; Zhang, Z.;Wang, J.; Cui, A.; Gao,Y. *Org. Biomol. Chem.* **2005**, *3*, 1387; (b) Turfan, B.; Akkaya, E. U. *Org. Lett*. **2002**, *4*, 2857

(44) (a) Bricks, J. L.; Kovalchuk, A.; Trieflinger, C.; Nofz, M.; Büschel, M.; Tolmachev, A. I.; Daub, J.; Rurack, K. *J. Am. Chem. Soc*. **2005**, *127*, 13522; (b) Wang, R.; Yu, F.; Liu, P.; Chen, L. *Chem. Commun*. **2012**, *48*, 5310

(45) (a) Li, L. *Crit Rev Oral Biol Med*. **2004**, *14*, 100; (b) Aoba, T.; Fejerskov, O. *Crit. Rev. Oral Biol. Med*. **2002**, *13*, 155; (c) Ashby, M. T. *J. Dent. Res.* **2010**, *34*, 1

(46) (a) Cao, X.; Lin, W.; Yu, Q.; Wang, J. *Org. Lett*. **2011**. *13*, 6098; (b) Lv, Y.; Xu, J.; Guo, Y.; Shao, S. *J. Incl. Phenom. Macrocycl. Chem*. **2012**, *72*, 95

(47) (a) Guliyev, R.; Buyukcakir, O.; Sozmen, F.; Bozdemir, O. A. *Tetrahedron. Lett*. **2009**, *50*, 5139; (b) Lee, C.; Yoon, H.; Shim, J.; Jang, W. *Chem. Eur. J.* **2012**, *18*, 4513

(48) Li, Q.; Guo, Y.; Shao, S. *Analyst* **2012**, *137*, 4497

(49) Coskun, A.; Baytekin, B. T.; Akkaya, E. U. *Tetrahedron Lett*. **2003**, *44*, 5649

(50) (a) Araujo, A. N.; Couto, C. M. C. M.; Lima, J. L. F. C.; Montenegro, M. C. B. S. M. *J. Agric. Food Chem.* **1998**, *46*, 168; (b) Lowinsohn, D.; Bertotti, M. *Food. Addit. Contam*. **2001**, *18*, 773

(51) Gu, X.; Liu, C.; Zhu, Y. C.; Zhu, Y. Z. *J. Agric. Food Chem*. **2011**, *59*, 11935

(52) Thannickal, V. J.; Fanburg, B. L. *Am J Physiol Lung Cell Mol Physiol*, **2000**, *279*, L1005

(53) Apel, K.; Hirt, H. *Annu. Rev. Plant Biol*. **2004**, *55*, 373

(54) Turrens, J. F. *J. Physiol*, **2003**, 552, 335

(55) Sekiya, M.; Umezawa, K.; Sato, A.; Citterio, D.; Suzuki, K. *Chem. Commun*. **2009**, *7*, 3047

(56) Sun, Z.; Liu, F.; Chen, Y.; Tam, K. H.; Yang, D. *Org*. *Lett*. **2008**, *10*, 2171

(57) Benniston, A. C.; Copley, G.; Elliott, K. J.; Harrington, R. W.; Clegg, W. *Eur. J. Org. Chem*. **2008**, *16*, 2705

(58) (a) Trachootham, D.; Lu, W. Q.; Ogasawara, M. A.; Valle, N. R. D.; Huang, P. *Antioxid. Redox. Signaling*. **2008**, *10*, 1343; (b) Giannoni, E.; Buricchi, F.; Grimaldi, G.; Parri, M.; Cialdai, F.; Taddei, M. L.; Raugei, G.; Ramponi, G.; Chiarugi, P. *Cell. Death. Differ*. **2008**, *15*, 867

(59) Sun, Z.; Wang, H.; Liu, F.; Chen, Y.; Tam, K. H.; Yang, D. *Org*. *Lett*. **2009**, *11*, 1887

(60) (a) Pacher, P.; Beckman, J. S.; Liaudet, L. *Physiol. Rev.* **2007**, *87*, 315; (b) Stamler, J. S.; Singel, D. J.; Loscalzo, J. *Science*. **1992**, *258*, 1898

(61) Ledo, A.; Frade, J.; Barbosa, R. M.; Laranjinha, J., *Mol*. *Aspects*. *Med*., **2004**, *25*, 75

(62) (a) Gabe, Y.; Urano, Y.; Kikuchi, K.; Kojima, H.; Nagano, T. *J. Am. Chem. Soc*. **2004**, *126*, 3357; (b) Zhang, X.; Chi, R.; Zou, J.; Zhang, H. *Spectrochimica Acta*: Part A **2004**, *60*, 3129; (c) Gabe, Y.; Ueno, T.; Urano, Y.; Kojima, H.; Nagano, T. *Anal. Bioanal. Chem*. **2006**, *386*, 621; (d) Miller, E. W.; Chang, C. J. *Curr. Opin. Chem. Biol.* **2007**, *11*, 620

(63) (a) Dejam, A.; Hunter, C. J.; Schechter, A. N.; Gladwin, M. T. *Blood. Cell. Mol. Dis*. **2004**, *32*, 423; (b) Gladwin, M. T. *J. Clin. Invest.* **2004**, *113*, 19

(64) Li, M.; Wang, H.; Zhang, X.; Zhang, H. *Spectrochimica Acta*: *Part A* **2004**, *60*, 987

(65) Waddell, W. J.; Bates, R. G. *Physiol. Rev.* **1969**, *49*, 285

(66) Boens, N.; Leen, V.; Dehaen, W. *Chem. Soc. Rev*. **2012**, *41*, 1130

(67) (a) Boens, N.; Leen, V.; Dehaen, W. *Chem. Soc. Rev*. **2012**, *41*, 1130; (b) Gareis, T.; Huber, C.; Wolfbeis, O. S.; Daub, J. *Chem. Commun.* **1997**, *18*, 1717; (c) Kollmannsberger, M.; Gareis, T.; Heinl, S.; Breu, J.; Daub, J. *Angew. Chem. Int. Ed.* **1997**, *36*, 1333; (d) Baruah, M.; Qin, W.; Basaric, N.; Borggraeve. W. M. D.; Boens, N. *J. Org. Chem*. **2005**, *70*, 4152

(68) (a) Ying, L. Q.; Branchaud, B. P. *Bioorg. Med. Chem. Lett*. **2011**, *21*, 3546; (b) Urano, Y.; Asanuma, D.; Hama, Y.; Koyama, Y.; Barrett, T.; Kamiya, M.; Nagano, T.; Watanabe, T.; Hasegawa, A.; Choyke, P. L.; Kobayashi, H. *Nat. Med.* **2009**, *15*, 104

(69) (a) Nicolas, D. C.; Lakowicz, J. R. *Tetrahedron. Lett*. **2001**, *42*, 9105; (b) Zhai, D.; Lee, S. C.; Vendrell, M.; Leong, L. P.; Chang, Y. T. *ACS Comb. Sci*. **2012**, *14*, 81

(70) (a) Kand, D.; Mishra, P. K.; Saha, T.; Lahiri, M.; Talukdar, P. *Analyst* **2012**, *137*, 3921; (b) Li, X.; Qian, S.; He, Q.; Yang, B.; Li, J.; Hu, Y. *Org. Biomol. Chem*. **2010**, *8*, 3627.; (c)Lu, J.; Sun, C.; Chen, W.; Ma, H.; Shi, W.; Li, X. *Talanta* **2011**, *83*, 1050.; (d) Shao, J.; Guo, H.; Ji, S.; Zhao, J. *Biosens. Bioelectron*. **2011**, *26*, 3012

(71) Lee, J. J.; Lee, S. C.; Zhai, D. T.; Ahn, Y. H.; Yeo, H. Y.; Tan, Y. L.; Chang, Y. T. *Chem. Commun*. **2011**, *47*, 4508

(72) (a) Parhi, A. K.; Kung, M. P.; Ploessl, K.; Kung, H. F. *Tetrahedron Lett*. **2008**, *49*, 3395; (b) Smith, N. W.; Alonso, A.; Brown, C. M.; Dzyuba, S. V. *Biochem. Biophys. Res. Commun*. **2010**, *391*, 1455; (c) Lee, J. S.; Kang, N.; Kim, Y. K.; Samanta, A.; Feng, S.; Kim, H. K.; Vendrell, M.; Park, J. H.; Chang, Y. T. *J. Am. Chem. Soc*. **2009**, *131*, 10077; (d) Ojida, A.; Sakamoto, T.; Inoue, M.; Fujishima, S.; Lippens, G.; Hamachi, I. *J. Am. Chem. Soc*. **2009**, *131*, 6543; (e) Ono, M. ; Ishikawa, M.; Kimura, H.; Hayashi, S.; Matsumura, K.; Watanabe, H.; Shimizu, Y.; Cheng, Y.; Cui, M.; Kawashima, H.; Saji, H. *Bioorg. Med. Chem. Lett*. **2010**, *20*, 3885

(73) (a) Kim, T. K.; Lee, D. N.; Kim, H. J. *Tetrahedron Lett*. **2008**, *49*, 4879; (b) Peng, H. J.; Chen, W. X.; Cheng, Y. F.; Hakuna, L.; Strongin, R.; Wang, B. H. *Sensors*. **2012**, *12*, 15907

(74) (a) Zhang, S.; Wu, T.; Fan, J. L.; Li, Z. Y.; Jiang, N.; Wang, J. Y.; Dou, B. R.; Sun, S. G.; Song, F. L.; Peng, X. J. *Org. Biomol. Chem*. **2013**, *11*, 555; (b) Kamkaew, A.; Lim, S. H.; Lee, H. B.; Kiew, L. V.; Chung, L. Y.; Burgess, K. *Chem. Soc. Rev.*, **2013**, *42*, 77; (c) Lim, S. H.; Thivierge, C.; Nowak-Sliwinska, P.; Han, J. Y.; van den Bergh, H.; Wagnieres, G.; Burgess, K.; Lee, H. B. *J. Med. Chem.*, **2010**, *53*, 2865; (d) Jiao, L.; Yu, C.; Uppal, T.; Liu, M.; Li, Y.; Zhou, Y.; Hao, E.; Hu, X.; Vicente, M. G. H., *Org. Biomol. Chem*, **2010**, *8*, 2517

(75) (a) Niu, S. L.; Massif, C.; Ulrich, G.; Ziessel, R.; Renardb, P. Y.; Romieu, A. *Org. Biomol. Chem*. **2011**, *9*, 66; (b) Chen, C. S.; Chen, W. N. U.; Zhou, M.; Arttamangkul, S.; Haugland, R. P. *J. Biochem. Biophys. Methods* **2000**, *42*, 137; (c) Talbot, S.; Turmel, P. T.; Liazoghli, D.; Sénécal, J.; Gaudreau, P.; Couture, R. *J. Neuroinflamm*. **2009**, *6*, 11; (d) Mikhalyov, I.; Olofsson, A.; Gröbner, G.; Johansson, L. B. A. *Biophys. J.* **2010**, *99*, 1510

(76) (a) Schade, S. Z.; Jolley, M. E.; Sarauer, B. J.; Simonson, L. G. *Anal. Chem.* **1996**, *243*, 1; (b) Rayo, J.; Amara, N.; Krief, P.; Meijler, M. M.*J. Am. Chem. Soc*. **2011**, *133*, 7469

(77) (a) Ehrenschwender, T.; Wagenknecht, H. A. *J. Org. Chem.* **2011**, *76*, 2301; (b) Gießler, K.; Griesser,H.; Göhringer, D.; Sabirov, T.; Richert, C. *Eur. J. Org. Chem*. **2010**, *19*, 3611; (c) Bandhuvula, P.; Li, Z.; Bittman, R.; Saba, J. D., *Biochem. Biophys. Res.*

Commun. **2009**, *380*, 366; (d) Takeji, T. E.; Ryoko, I., *Bioorg. Med. Chem. Lett*. **2011**, *21*, 4206

(78) (a) Goncalves, M. S. *Chem. Rev.* **2009,** *109*, 190; (b) Metzker, M. L.; Lu, J.; Gibbs, R. A. *Science* **1996**, *271*, 1420; (b) Lee, L. G.; Connell, C. R.; Woo, S. L.; Cheng, R. D.; McArdle, B. F.; Fuller, C. W.; Halloran, N. D.; Wilson, R. K. *Nucleic Acids Res*. **1992**, *20*, 2471; (c) Perronet, K.; Bouyer, P.; westbrook, N.; Soler, N.; Fourmy, N.; Yoshizawa, S. *J. Lumin*. **2007**, *127*, 264

(79) (a) Kliger, Y.; Gallo, S. A.; Peisajovich, S. G.; Munoz-Barroso, I.; Avkin, S.; Blumenthal, R. Shai, Y. *J. Biol. Chem*. **2001**, *276*, 1391; (b) Pap, E. H.; Drummen, G. P.; Post, J. A.; Rijken, P. J.; Wirtz, K. W. *Methods Enzymol*. **2000**, *319*, 603

(80) (a) Rogers, R. A.; Jack, R. M.; Furlong, S. T. *J. Cell Sci*. **1993**, *106*, 485; (b) Kamisaka, Y.; Noda, N. *J. Biochem*. **2001**, *129*,19; (c) Farber, S. A.; Olson, E. S.; Clark, J. D.; Halpern, M. E. *J. Biol. Chem*. **1999**, *274*, 19338; (d) Sachl, R.; Mikhalyov, I.; Gretskaya, N.; Olzynska, A.; Hof, M.; Johansson, L. B. A. *Phys. Chem. Chem. Phys*. **2011**, *13*, 11694

(81) (a) Yu, B.; Wright, S. D. *J. Biol. Chem*. **1996**, *271*, 4100; (b) Wurfel, M. M.; Wright, S. D. *J. Immunol*. **1997**, 158, 3925

(82) Namkung, W.; Padmawar, P.; Mills, A. D.; Verkman, A. S. *J. Am. Chem. Soc*. **2008**, *130*, 7794

(83) Gagne, A.; Banks, P.; Hurt, S. D. *J. Recept. Sig. Transd.* **2002**, *22*, 333

(84) (a) Crivellato, E.; Candussio, L.; Rosati, A. M.; Klugmann, F. B.; Mallardi, F.; Decorti, G. *J. Histochem. Cytochem.* **2002**, *50*, 731; (b) West, R.; Panagabko, C.; Atkinson, J. *J. Org. Chem*. **2010**, *75*, 2883; (c) McCusker, C.; Carroll, J. B.; Rotello, V. M. *Chem. Commun*. **2005**, *8*, 996; (d) Yee, M.; Fas, S. C.; Stohlmeyer, M. M.; Wandless, T. J.; Cimprich, K. A. *J. Biol. Chem.* **2005**, *280*, 29053

(85) Ziessel, R.; Bonardi, L.; Retailleau, P.; Ulrich, G. *J. Org. Chem*. **2006**, *71*, 3095

(86) Tyagarajan, K.; Pretzer, E.; Wiktorowicz, J. E. *Electrophoresis* **2003**, *24*, 2348

(87) Matsumoto, T.; Urano, Y.; Shoda, T.; Kojima, H.; Nagano, T. *Org. Lett*. **2007**, *9*, 3375

(88) Wang, C.; Xie, F.; Suthiwangcharoen, N.; Sun, J.; Wang, Q. *Science China* **2012**, *55*, 125

(89) Hoogendoorn, S.; Blom, A. E. M.; Willems, L. I.; Marel, G. A.; Overkleeft, H. S. *Org. Lett*. **2011**, *13*, 5656

(90) Ziessel R, Ulrich G, Harriman A. *New J. Chem.* **2007**, *31*, 496

(91) Wang, P.; Giese, R. W. *J Chromatogr*: *A* **1998**, *809*, 211

(92) Crawford, S. M.; Thompson, A. *Org. Lett*. **2010**, *12*, 1424

(93) Tram, K.; Twohig, D.; Yan, H. *Nucleos. Nucleot. Nucleic. Acids*. **2011**, *30*, 1

(94) (a) Doeberitz, M. V; Lacroix, J. *Cancer Metast Rev*. **1999**, *18*, 43; (b) Cerchia, L.; Hamm, J.; Libri, D.; Tavitian, B.; de Franciscis, V. *FEBS Lett.* **2002**, *528*, 12; (c) Gatto, B.; Palumbo, M.; Sissi, C. *Curr. Med. Chem.* **2009**, *16*, 1248

(95) (d) Nielsen, P. E.; Egholm, M. *Curr Issues Mol Biol*. **1999**, *1*, 89

(96) Ray, A; Norden, B. *Faseb. J*. **2000**, *14*, 1041

(97) Shakeel, S,; Karim, S.; Ali, A. *J. Chem. Technol. Biotechnol.*, **2006**, *81*, 892

(98) Hyrup, B.; Nielsen, P. E. *Bioorg. Med. Chem.*, **1996**, *4,* 5

(99) (a) Nielsen, P. E. *Mol. Biotechnol.* **2004**, *26*, 3; (b) Pooga, M.; Land, T.; Bartfai, T.; Langel, U. *Biomol. Eng*., **2001**, *17*, 6

(100) a) Macadangdang, B.; Zhang, N.; Lund, P. E.; Marple, A. H.; Okabe, M.; Gottesman, M. M.; Appella, D. H.; Kimchi-Sarfaty, C. *PLoS. ONE*, **2011**, *6*, e17981; b) Larsen, H. J.; Nielsen, P. E. *Nucleic Acids Res*., **1996**, 2*4,* 458

(101) Albert, U. L.; Mercedes, A.; Fernando, A. *Chem. Rev.* **2009**, *109*, 2455

(102) Falkiewicz, B.; Wisniowski, W.; Kolodziejczyk, A. S.; Wisniewski, K. *Nucleosides, Nucleotides. Nucleic Acids*, **2001**, *20*, 1393

(103) (a) Fader, L. D.; Boyd, M.; Tsantrizos, Y. S. *J. Org. Chem*. **2001**, *66*, 3372; (b) Kofoed, T.; Hansen, H. F.; Orum, H.; Koch, T. *J. Peptide Sci.* **2001**, *7*, 402

(104) Egholm, M.; Nielsen, P. E.; Buchardt, O.; Berg, R. H. *J. Am. Chem. Soc.* **1992**, *114*, 9677

(105) Verheijen, J. C.; Grotenbreg, G. M.; de Ruyter, L. H.; van der Klein, P. A. M.; van der Marel, G. A.;van Boom, J. H. *Tetrahedron Lett*. **2000**, *41*, 3991

(106) Thomson, S. A.; Josey, J. A.; Cadilla, R.; Gaul, M. D.; Hassman, C. F.; Luzzlo, M. J.; Pipe, A. J.; Reed, K. L.; Ricca, D. J.; Wiethe, R. W.; Noble, S. A. *Tetrahedron.* **1995**, *51*, 6179

(107) Viirre, R. D.; Hudson, R. H. E. *J. Org. Chem*. **2003**, *68*, 1630

(108) (a) Huang, P. C.; Hsu, G. J.; Zhuang, B. R.; Sung, K. *Amino Acids*, **2008**, *34*, 449; (b) Sung, K.; Chen, F. L.; Huang, P. C. *Synlett*. **2006**, *16*, 2667; (c) Maison, W.;

Schlemminger, I; Westerhoff, O.; Martens, J. *Bioorg. Med. Chem*. **2000**, *8*, 1343; (d) Xu, P.; Zhang, T.; Wang, W.; Zou, X.; Zhang, X.; Fu, Y. *Synthesis*. **2003**, *8*, 1171

(109) Porcheddu, A.; Giacomelli, G. *Curr. Med. Chem.* **2005**, *12*, 2561

(110) Brian, D.; Gildea, B. D.; Casey, S.; MacNeill, J.; Perry, O. H.; Ditte, S. D.; Coull, J. M. *Tetrahedron Lett*. **1998**, *39*, 7255

(111) Hyrup, B.; Egholm, M.; Buchardt, O.; Nielsen, P. E. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1083

(112) (a) Wittug, P.; Kajanus, J.; Edwards, K.; Nielsen, P. E.; Nordén, B.; Malmstom, B. G. *FEBS Lett*. **1995**, *365*, 27; (b) Demidov, V.V.; Potaman, V.N.; Frank-Kamenetskii, M. D.; Egholm, M.; Buchardt, O.; Sönnichsen, S. H.; Nielsen, P. E. *Biochem. Pharmacol*. **1994**, *48*, 1310; (c) Koppelhus, U.; Nielsen, P. E. *Adv. Drug. Deliv. Rev*. **2003**, *55*, 267

(113) (a) Ge, R.; Heinonen, J. E.; Svahn, M. G.; Mohamed, A. J.; Lundin, K. E.; Smith, C. I. E. *FASEB J*., **2007**, *21*, 1902; (b) Larsen, H. J.; Nielsen, P. E. *Nucleic Acids Res*. **1996**, *24*, 458; (c) Belotserkovskii, B. P.; Liu, R.; and Hanawalt, P. C. *Mol. Carcinog*. **2009**, *48*, 299

(114) (a) Lecosnier, S.; Cordier, C.; Simon, P.; François, J. C.; Behmoaras, T. E. S. *FASEB J*. **2011**, *25*, 2001; (b) Hamoudi, F. B.; Leforestier, E.; Beaufort, C. S.; Nielsen, P. E.; Giovannangeli, C.; Behmoaras, T. E. S. *Nucleic Acids Res*. **2007**, *35*, 3907; (c) Dias, N.; Beaufort, C. S.; Forestier, E.; Auvin, C.; Hélène, C.; Behmoaras, T. E. S. *J. Mol. Biol*. **2002**, *320*, 489; (d) Sun, L.; Fuselier, J. A.; Murphy, W. A.; Coy, D. H. *Peptides* **2002**, *23*, 1557

(115) (a) Kaushik, N.; Pandey, V. N. *Virology* **2002**, *303*, 297; (b) Ahn, D. G.; Lee, W. L.; Choi, J. K.; Kim, S. J.; Plant, E. P.; Almazán, F.; Taylor, D. R.; Enjuanes, L.; Oh, J. W. *Antiviral Res.* **2011**, *91*, 1; (c) Arzumanov, A.; Walsh, A. P.; Rajwanshi, V. K.; Kumar, R.; Wengel, J.; Gait, M. J. *Biochemistry* **2001**, *40*, 14645; (d) Taylor, R. W.; Chinnery P. F.; Turnbull, D. M.; Lightowlers, R. N. *Nat. Genet*. **1997**, *15*, 212

(116) Dias, N.; Stein, C. A. *Mol. Cancer Ther***. 2002**, *1*, 347

(117) (a) Chen, G.; Zhang, C.; Zhang, B.; Wang, G.; Lu, D.; Xu, Z.; Yan, P. *PLoS ONE* **2011**, *6*, 25527; (b) Blanco, A M.; Artero, R. *Methods* **2010**, *52*, 343

(118) (a) Nielsen, P. E. Ed. Peptide Nucleic Acids : Methods and Protocols (11th ed.); Humana Press Inc., Totowa, NJ, **2002**, pp. 148-150; (b) Murdock, D. G., Christacos, N. C., and Wallace, D. C. *Nucleic Acids Res*. **2000**, *28*, 4350

(119) a) Kam, Y.; Rubinstein, A.; Nissan, A.; Halle, D.; Yavin, E. *Mol. Pharm*., **2012**, 9, 685; b) Totsingan, F.; Tedeschi, T.; Sforza, S.; Corradini, R.; Marchelli, R. *Chirality*, **2009**, *21*, 245

(120) (a) Veselkov, A. G., Demidov, V., Nielsen, P. E., Frank-Kamenetskii, M. D. *Nucleic Acids Res*. **1996**, *24*, 2483; (b) Nordén, B.; Ray, A. *FASEB J.* **2000**, *14*, 1041

(121) Kohn, M.; Breinbauer, R. *Angew. Chem. Int. Ed*. **2004**, *43*, 3106

(122) Schilling, C. I.; Jung, N.; Biskup, M.; Schepers, U.; Brase, S. Chem. Soc. Rev., **2011**, *40*, 4840]

(123) (a) Castro, A.; Williams, J. *Anal. Chern*. **1997**, *69*, 3915; (b) Tanke, H.; Wiegant, J.; van Gijlswijk, R.; Bezrookove, V.; Pattenier, H.; Heetebrij, R. *Eur. J. Human Gen*. **1999**, *7*, 2; (c) Wiegant, J.; van Gijlswijk, R.; Heetebrij, R.; Bezrookove, V.; Raap, A.; Tanke, H. *Cytogenet. Cell Genet*. **1999**, *87*, 47; (d) Alers, J.; Rochat, J.; Krijtenburg, P.; van Dekken, H.; Raap, A.; Rosenberg, C. *Gene. Chromosome. Cane*. **1999**, *25*, 301

(124) Tram, K.; Twohig, D.; Yan, H. *Nucleos. Nucleot. Nucl*. **2011**, *30*, 1

(125) (a) Crawford, S. M.; Thompson, A. *Org Lett*. **2010**, *12*, 1424-1427; (b) Goeb, S.; Ziessel, R. *Tetrahedron Lett*. **2008**, *49*, 2569

(126) Li, L.; Nguyen, B.; Burgess, K. *Bioorg. Med. Chem. Lett*. **2008**, *18*, 3112

(127) Gabe, Y.; Ueno, T.; Urano, Y.; Kojima, H.; Nagano, T. *Anal. Bioanal. Chem*. **2006**, *386*, 621

(128) Goze, C.; Ulrich, G.; Mallon, L. J.; Allen, B. D.; Harriman, A.; Ziessel, R. *J. Am. Chem. Soc.* **2006**, *128*, 10231

(129) Led, J. J.; Gesmar, H. *Chem Rev*. **1991**, *91*, 1413

(130) Davies, W. C.; Evans, G. B.; Hulbert, F. L. *J. Chem. Soc.* **1939**, 412

(131) (a) Böhme, H.; Boll, E. *Chem. Ber.* **1957**, *90*, 2013; (b) Wright, D. A.; Wulff, C. A. *J. Org. Chem.* **1970**, *35*, 4252; (c) Nevstad, G. O.; Songstad, *J. Acta Chem. Scand. B*. **1984**, *38*, 469

(132) Jones, L. W.; Whalen, H. F. *J. Am. Chem. Soc.* **1925**, *47*, 1343

(133) (a) Mills, J. E.; Maryanoff, C. A.; Cosgrove, R. M.; Scott, L.; McComsey, D. F. *Org. Prep. Proced. Int.* **1984**, *16*, 97; (b) Mills, J. E.; Maryanoff, C. A.; McComsey, D. F.; Stanzione, R. C.; Scott, L. *J. Org. Chem.* **1987**, *52*, 1857; (c) Souquet, F.; Martens, T.; Fleury, M.-B. *Synth. Commun*. **1993**, *23*, 817

(134) (a) Hon, Y. S.; Chou, Y. Y.; Wu, I. C. *Synth. Commun*. **2004**, *34*, 2253; (b) Matsumoto, K. *Angew. Chem., Int. Ed.*. **1982**, *94*, 922; (c) Hon, Y. S.; Hsu, T. R.; Chen, C. Y.; Lin, Y. H.; Chang, F. J.; Hsieh, C. H.; Szu, P. H. *Tetrahedron*. **2003**, *59*, 1509; (d) Miyano, S.; Mori, A.; Hokari, H.; Ohta, K.; Hashimoto, H. *Bull. Chem. Soc. Jpn*. **1982**, *55*, 1331

(135) Lacowicz, J. R. *Principles of Fluorescence Spectroscopy***,** *3rd ed*.; Springer: New York, **2006**, 54

(136) Helms, A.; Heiler, D.; Mclendon, G. *J. Am. Chem. Soc.* **1992**, *114*, 6221

(137) Leen, V.; Leemans, T.; Boens, N.; Dehaen, W. *Eur. J. Org. Chem.*, **2011**, *23*, 4386

(138) Liras, M.; Prieto, J. B.; Pintado-Sierra, M.; Arbeloa, F. L.; Garcia-Moreno, I.; Costela, A.; Infantes, L.; Sastre, R.; Amat-Guerri, F. *Org. Lett.*, **2007**, *9*, 4183

(139) Saxon, E.; Bertozzi, C. R. *Science* **2000**, *287*, 2007

(140) Nilsson, B. L.; Kiessling, L. L.; Raines, R. T. *Org. Lett*. **2001**, *3*, 9

(141) Mula, S.; Ray, A. K.; Banerjee, M.; Chaudhuri, T.; Dasgupta, K.; Chattopadhyay, S. *J. Org. Chem.* **2008**, *73*, 2146

(142) May, D. A.; Lash, T. D. *J. Org. Chem.*, **1992**, *57*, 4820

(143) Yukitami, M.; Takuzo Aida.; Kentaro, Y. *J. Am. Chem. Soc*. **2000**, *122*, 5278

(144) Noeth, H.; Vahrenkamp, H. *J. Organomet. Chem.* **1968**, *11*, 399

(145) Schwergold, C.; Depecker, G.; Giorgio, C. D.; Patino, N.; Jossinet, F.; Ehresmann, B.; Terreux, R.; Cabrol-Bass, D.; Condom, R. *Tetrahedron*, **2002**, *58*, 5675

(146) Muller, D.; Zeltser, I.; Bitan, G.; Gilon, C. *J. Org. Chem.* **1997**, *62*, 411

(147) Da Ros, T.; Bergamin, M.; Vazquez, E.; Spalluto, G.; Baiti, B.; Moro, S.; Boutorine, A.; Prato, M. *Eur. J. Org. Chem.*, **2002**, *3*, 405